

The Journal of Laboratory and Clinical Medicine

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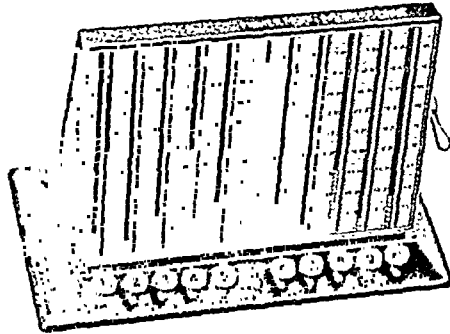
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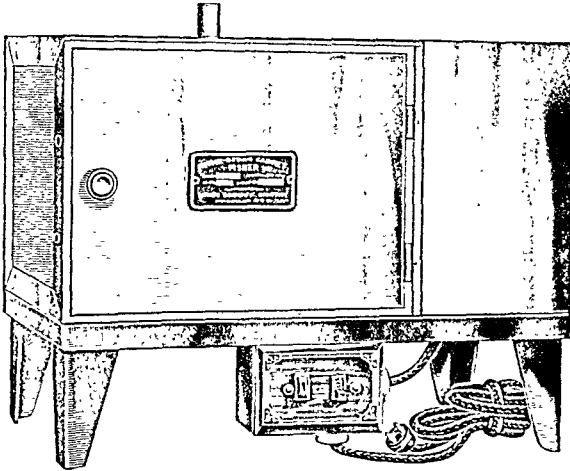
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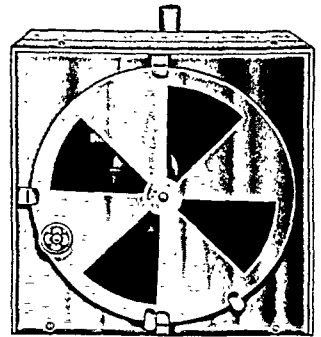
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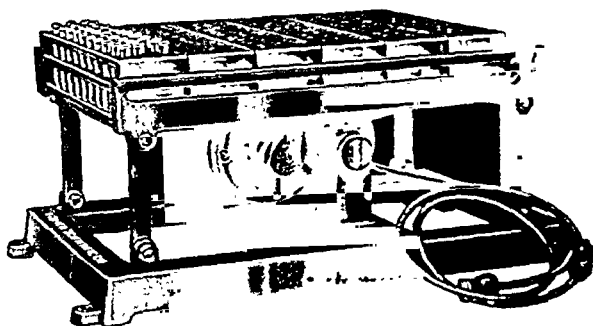
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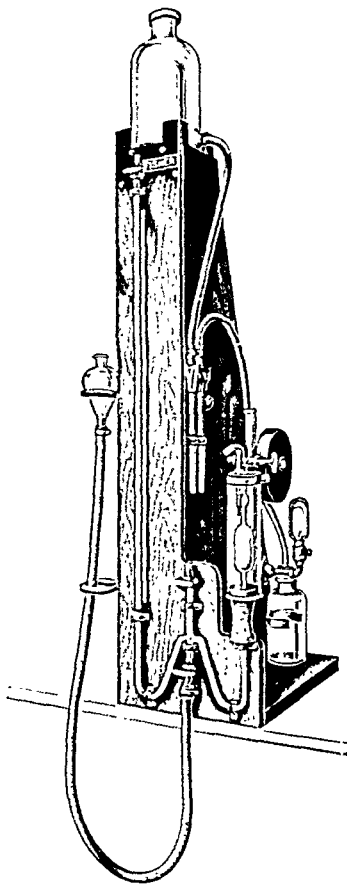
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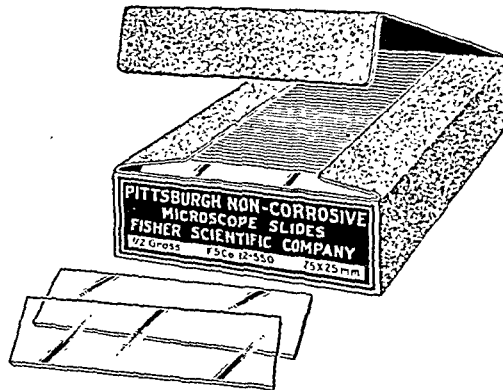
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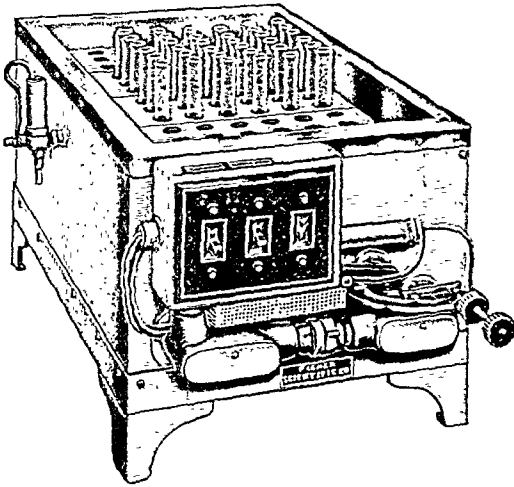
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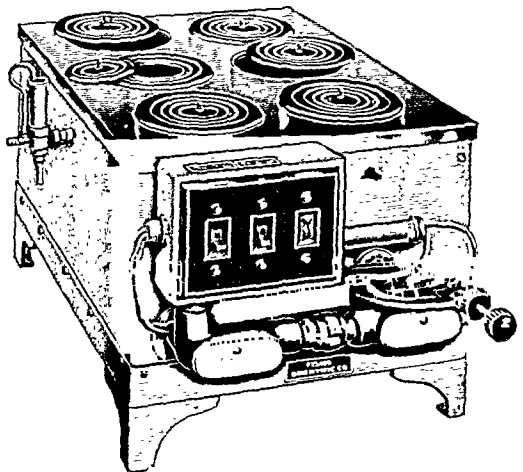
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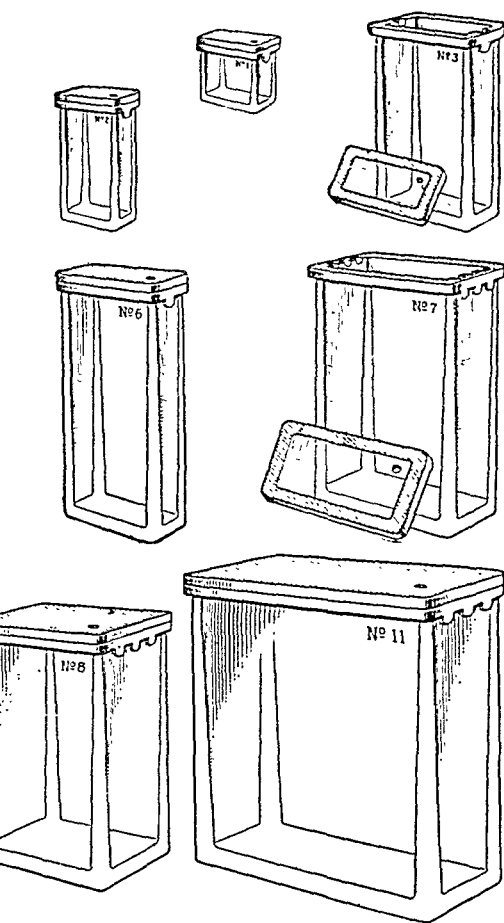
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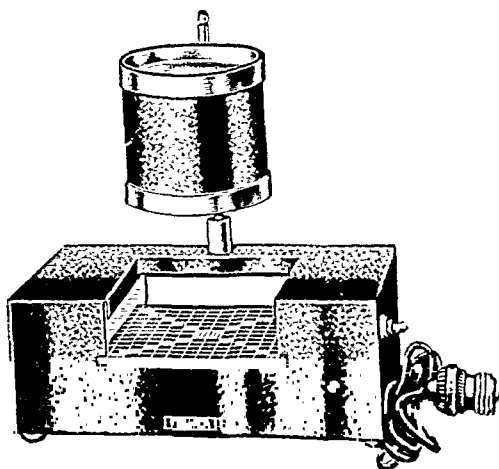
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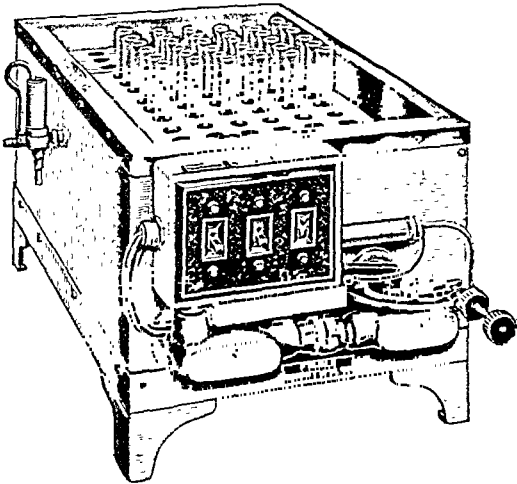
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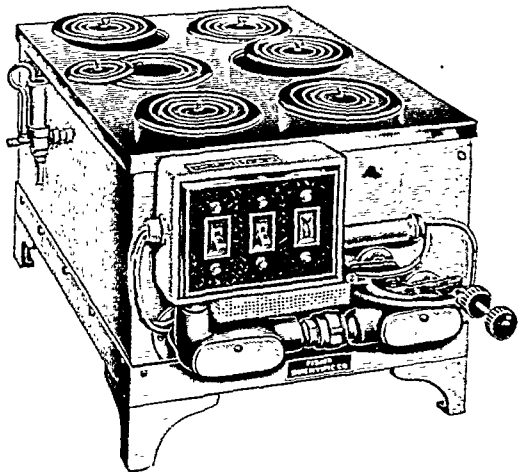
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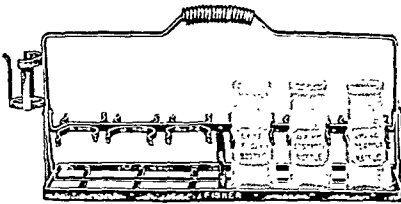
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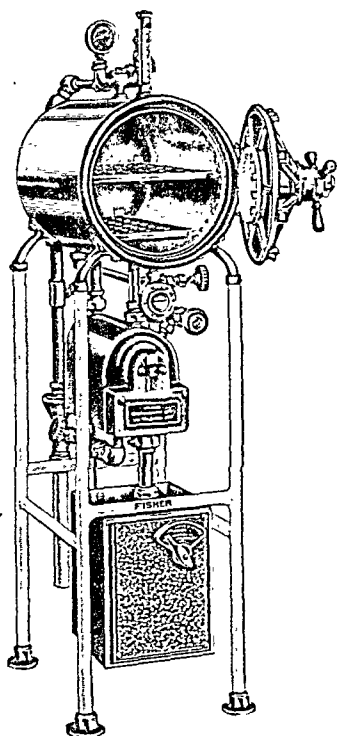
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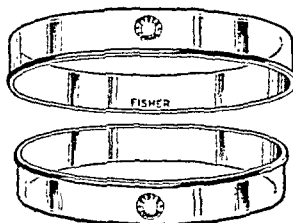
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VOL. XIX

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No. 1

CLINICAL AND EXPERIMENTAL

THE MEDICINE OF THE AMERICAN INDIAN*

HARLOW BROOKS, M.D., NEW YORK CITY, N. Y.

AS PRACTITIONERS of medicine we are in this day perhaps too prone to forget the value to medicine of the work of those who have preceded us, perhaps too much persuaded that modern medicine and modern science are only of value. We forget too easily that medicine like most other things is a matter of evolution and that most of our knowledge has in fact been based on a foundation of the experience and study of ages. I wonder sometimes whether we appreciate fully how absolutely dependent we are in medicine on what has gone before. How many of us are competent to practice medicine even today on the frontier away from the manufacturing pharmacist, away from our colleagues, from our journals, and from the basic facts in particular which have evolved from the studies of centuries past.

My observation has been that the modern scientifically trained student is in large degree more and more dependent on the facts and experiences which others have gleaned. Many, if not most of our research workers must base their investigations on a foundation of evolved knowledge which we take altogether too casually and with too little respect for its origin.

How many of us are competent to even set a fracture or to treat a serious wound away from the detailed assistance of the hospital and the carefully worked out preparations which we owe to others. Briefly how many of us with all our present knowledge might successfully practice the art of physic

*An address before the Richmond Academy of Medicine, Richmond, Virginia November 22, 1932.

Received for publication, December 5, 1932.

in the stone age, entirely without the assistance which others render to us? If one but thinks of this, better if one tries to meet the emergencies of practice away from drug stores, away from hospitals, without the microscope, and without the x-ray, how would we get on?

Hence it seems to me that it is well for us to study medical evolution, to interest ourselves in the pregnant subject of medical history, to learn of, as we shall certainly come to respect our predecessors in the practice of medicine and surgery. To do this one must acquire more than a passing knowledge of archeology and ethnology; one must delve sincerely into the customs and requirements of ancient peoples. Such a study is certain to broaden our knowledge and certain also to create in us a great respect for those men and peoples who with little inherited knowledge and no literature have none the less laid the foundations of medical art and science.

How many of us appreciate what we owe to those who gave us mercury, iodides, the salicylates, digitalis, the saline laxatives, cascara and aloes, quinine, cocain, alcohol, and the sedatives of the opium group? These and many other drugs on which we daily depend were discovered and properly used by the red man. Thousands of years before Witherington was born, the Indian of the southwest used infusions of Foxglove in heart disease, and if history is to be relied upon, he used it properly.

Another point which we must always bear in mind in these studies is that modern medicine as we term it could not as it now stands have been adapted to the needs of ancient man, chiefly because modern medicine is now dependent absolutely on the assistance of the chemist, the physicist, the bacteriologist, the pharmacist, and the manufacturer. The medicine of the stone age was necessarily also of the stone age; the electric knife was not available but the keen edged flake of obsidian was, and for that surgeon who is high-minded of his present-day skill, permit me to recommend that he try in his work only such tools as the stone age supplied, and then compare his results and skill with those of the surgeon of that day. If we will properly appreciate the medicine of primitive man, we must know his needs and the facilities furnished him; they were indeed very different from those of this day.

Perhaps as a class we less appreciate the history and the archeology of our native Americans than those of any other people. All of us know of ancient Egypt, of Rome, of Greece; we are, as properly educated men, tolerably familiar with those ancient peoples, and we know a good deal of their medicine and of the basic knowledge which they evolved on which modern medicine depends absolutely and hourly. How many of us realize the fact that here in America we had a civilization which exceeded and preceded in art, mathematics, astronomy, architecture, agriculture, politics, and which certainly equalled even in theology that of any of the ancient peoples of Europe.

In Bolivia still stands, in spite of Spanish cupidity and religious intolerance, the devastated fragments of a city, greater in its architecture and in its sculpture than anything that Greece or Italy ever produced, and it preceded

the culture of these countries by at least several thousand years. How many of us realize that when the Spaniard came to America he found a civilization in certain areas which exceeded his own of that very time except in the two particulars of military organization and in navigation. For those of you who are skeptics, may I recommend that you read Mason's "Columbus Came Late," or still better for the more serious minded and studious of you, read Verrill's "Old Civilizations of the New World." For those of you who still doubt, instead of going on your next vacation to Greece or Egypt, go to Tia-huanaco in Bolivia, to Cusco, to Yucatan, Guatamala, or Honduras. Study in the museums of Lima or in the easily accessible and marvelous collections of the City of Mexico, or if still in doubt precede your own explorations by a visit to the Museum of the American Indian, Heye Foundation here in your own city. Many beautiful and marvelous things of ancient American origin are also to be seen any day in the American Museum of Natural History, any one of which must convince you that here in our own America we possessed a civilization and an art more marvelous than anything of its kind in Africa or Europe.

With this introduction permit me then to talk with you for a few moments concerning the medicine of the American Indian. At the outset it is important for you to understand that under the heading of the American Indian, a tremendous field is included. To conceive of the American Indian as a single race or people is as absurd as to speak of the Europeans as a single class group. The American Indian varies as widely in his tribal civilization standards and types as does the Italian from the Iclander. What I have to say to you this evening is chiefly of certain of the tribes with which I have been most familiar, of the Ojibways, the Sioux, the Blackfeet, the Apache, the Navaho, and something of the Pueblo people. I thus limit myself because I know so very much less of the medicine of the South American Indian including the tribes of Mexico, Honduras, Guatamala, Chili, Peru, Bolivia, of the Myas, the Guiamacs, even of the Aztecs and Preaztecs; and yet we know that these tribes exceeded in civilization, art, agriculture, architecture, sculpture, and astronomy the tribes of which I am to speak mostly tonight as much as the modern Englishman or German exceeds the Laplander or Esquimo of today. Of these tribes have sprung such heroes, statesmen as Tecumseh, as Bolivar, Pofiero Diaz, Cailles, Rubio, and others who are truly world figures and not merely citizens of one land or one people. Occasionally, as in comparison, I shall refer to the medicine of the southern Indian, drawing for my knowledge on the little that I have seen and from what I have read.

Also in passing I should like to say that inevitably the white man has lowered and befouled the Indian of whatever tribe by his religion and by his military methods. In these characteristics only, it seems to me, have we outdone the Indian; these have been the two channels through which we have "civilized" the red man.

Before it is possible to go on, may I tell you something of what the red man, whose medicine I am to speak to you of this evening, was. Perhaps I

can do no better than to quote at the outset what Colden says of the Iroquois, our predecessors in New York.*

On the death of Lieut. Governor de Laney in 1760, Colden came to New York as Governor; he was appointed Lieut. Governor in 1761. He produced a great mass of geographical, metaphysical, medical, and theological learning and literature. He was the son of a Scotch clergyman and was designed for the clergy, but he took up finally the study of medicine. He practiced after his graduation in England, for some time in Pennsylvania, and later, because of his distinction, he became surveyor general of the colony in New York. He was made a master in Chancery and received a large grant of land near the town of Montgomery in Orange County. He was regarded in England as the best informed man in the New World on the affairs of the British colonies in America. He was an early observer of the influence of climate on health. He was just and fair to the Indians, was much honored by them and was adopted by the Mohawks of Canajoharie. The books cited were apparently written to inform Great Britain on the Indian question and to stress the strength and importance of the Iroquois Federation with which he was keenly informed and sympathetic. He died at Spring Hill, near Flushing, on the twenty-first of September, 1776. His many manuscripts are safe in the archives of the New York Historical Society but little is commonly known of him now though he was for his times one of the most learned in electricity, in natural philosophy, and in history. He was the valued associate of Franklin, a correspondent of Linnaeus, Grovius and with other eminent authorities in the natural sciences. He was always keenly alive to the scientific medicine of the day though his important executive obligations required his giving up medical practice.

He says: "The Five Nations are a poor and generally called barbarous people, bred under the darkest ignorance; and yet a bright and noble genius shines through these black clouds." "None of the greatest Roman Heroes have discovered a greater love to their country or a greater Contempt of Death than these People called Barbarians have done, when Liberty came in Competition. Indeed I think that our Indians have outdone the Romans in this Particular; some of the greatest of those have we know murdered themselves to avoid Shame or Torments; but our Indians have refused to die meanly, or with but little pain when they thought their country's Honor would be at stake by it; but have given their bodies willingly to the most cruel of torments of their Enemies, to shew as they said, that the Five Nations consisted of Men, whose Courage and Resolution could not be shaken."

"Instead of virtues we have only taught them Vices, that they were entirely free from before that time." "If care were taken to implant and cultivate in them that general benevolence to Mankind, which is the first true principle of Virtue—They would no longer deserve the Name of Barbarians, but would become a people, whose Friendship might add Honor to the British Nation." "Their great Men, both Sachems and Captains, are gener-

*The History of the Five Indian Nations of Canada which are dependent on the Province of New York, and are barrier between the English and the French in that part of the World. By Hon. Cadwallader Colden. New Amsterdam Book Company, New York, 1902.

ally poorer than the common People; for they effect to give away and distribute all Presents or Plunder they get in their Treties or in War, so as to leave nothing to themselves."

The only writings which I have been able to secure of Governor Colden are concerning the history of the Indian wars and of their treaties. I have obtained nothing dealing with Indian Medicine. It is to be much regretted that a person of his medical training and scientific thought should not have written of the medical customs of these our predecessors in the medical practice of New York. It is very evident that he thoroughly respected and even admired these men. As to their political sagacity, trustworthiness and valor, he leaves little unsaid and where he recounts their cruelty and brutality, he is wont to compare it without honor to the white man with his religious persecutions and his retaliatory wars.

Clark Wissler of the American Museum of Natural History, one of our greatest living authorities on archeology and ethnology says (The American Indian): that the Indian stands out in his culture in sharp contrast to that of the old world, and that while we have replaced him in this land, we have absorbed a great deal of his culture, and that we have wrought great havoc by his withdrawal from painting, sculpture, and the decorative arts. This loss, though considerable would be small comparable to that if we were to lose from our economic life maize, cocoa, quinine, tobacco, potatoes, beans, squash, melons, and tomatoes, and what has evolved from our adaptation of their agricultural methods in the world. Irrigation, at least in this country is founded on Indian methods and procedures and their agricultural methods from the use of irrigation are still of immeasurable value to us today. I have seen beautiful irrigation systems installed by the prehistoric Indian, still in use today in Arizona, monumental systems which existed long before the advent of the white man. Wissler further says: "Imagine what would be lost to us if we could strike from our history, geography and literature all that pertains to the Indian!" The white man in many localities added but little beside iron and a more schooled and ruthless method of warfare.

Even our language, here in the new world has been in very large part taken from that of our red predecessors: we "hit the trail," we "pass over the divide," the camp fire, and the lodge circle have become endeared terms of our idiom, and a large number of us, in spite of our religious teachings still yearn to reach the "Happy hunting grounds." Our geographic names are in greater part adapted from those of the red man, our great railway systems, even our modern automobile highways for the greater part still follow the moccasin beaten pathways of the Indian, and most of our mannerisms and idioms which are typically American, evidence the survival in our culture, that of the red man. Colloquialisms and dramatic figures of American slang, poetry, and literature still portray the persistence and dramatic quality of the Indian orators, heroes, and statesmen.

In government, and particularly in the more idealistic forms expressed in altruistic communism, the world has never equalled the systems of the Pueblo peoples or that probably in vogue among the Mayas, Astecs, and

Toltecs. The League of the Five Nations in our own state of New York still represents a high water mark of organization and mutual consideration in governmental affairs. It was truly a league of nations, with ability, altruism, truth, and honesty in act as in theory.

I have already mentioned the deep impress which the Indian has made on our language. Notwithstanding the fact that the Indian peoples in the Americas have differed tremendously in traits, characteristics, in culture and in customs, as widely certainly as now exists between any of the civilized peoples of the whole world, still they possessed and still do, a mutual method of communication in the sign language which was almost universally understood and employed. It has interested me tremendously to note the strong similarity between this sign language of the Indians and that which our deaf and dumb now use. An Indian skilled in sign language, I am convinced would have little difficulty in understanding much or most of the sign language employed by the deaf and dumb, and the art of pantomime has never been more fully and beautifully demonstrated than by the orators of our primitive people.

In art, we have rarely as yet equalled that of the Indian, particularly in textiles exemplified by the beautiful and useful weaving of the South American tribes and the exquisite, when uncontaminated by white design, work of our Navahoes and Pueblos. We can never hope to equal the Indian in basketry, especially the work of the Pimas, Apache, and Supai, and the work of the ancient basket maker was never equalled, by even these artisans. In ceramics we can justly compare Indian antique work with that of the Chinese, for they have far excelled the Greeks in this work as well as in sculpture.

It is entirely inconceivable that such a people should not have had and practiced a system of medicine and surgery, comparable with their culture in other fields. The more we come to study their methods, the more do we become impressed with their skill and learning in these directions, especially in the field of traumatic surgery with which, naturally from the requirements of their habits of life, warfare and culture, they came most frequently in need.

Though I have been a student of Indian medicine since my early boyhood I know but little of the subject, but that little has created in me a feeling of great respect and admiration. Pioneer medicine in this country has been almost entirely derived from Indian methods, and I think that before I am done this evening I shall cause most of you to feel but honor and respect for the medicine of the American Indian. There are few methods of modern medicine which were not represented in kind, if not in precise technic by Indian methods, and we owe entirely to our Indian predecessors in the profession many of our most potent methods and drugs. Cesarean section, symphysiotomy, and the Credé method of placental expulsion, Verrill states (private communication) were practiced among certain of the more highly cultured tribes as those of certain districts in South and Southern America, long before Caesar was born. The same authority, corroborated by Hrdlicka and many other students of the subject, calls attention to the frequency and success of trephining of the skull and the use of artificial limbs ages before

the white man or even the Egyptians had advanced beyond the most primitive methods of medical or surgical procedure.

It is a curious and very unfortunate fact that the voluminous literature concerning Indian medicine and surgery has so very little to say concerning the practices of our most advanced and intelligent tribes, notably those of South America, of Central America and Mexico, where we know the highest types of civilization flourished, then present in the entire world. There are several reasons for this unfortunate state. Among these are the fact that most of the studies of these most advanced tribes have been made by ethnologists and archeologists who have had but little medical interest or schooling. Until we have discovered a Rosetta Stone which will unlock the secrets of the abundant records and codices of these peoples which we possess, we can expect to know only that of the medical and surgical practices of these people which has been handed down to the present descendants of these people. Yet we know that these tribes employed anesthetics for surgical procedures, notably cocaine, peyote and other drugs while the white man was a barbarian who put his wounded in war to death by "mercifully slitting the wezand."

Concerning the medical methods of our northern tribes we have much more exact knowledge from the methods of pioneer medicine which have been handed down to us and very largely also because many of the early missionaries, who were themselves more or less medically trained have recorded their observations as in the records of the Jesuit fathers. Also in very large part because the medical officers of our little army have from the very first been interested in the subject and have recorded their studies and observations in official reports. Furthermore we must accord great credit to our ethnologists and archeologists in North America who have seriously interested themselves constantly in these important matters. Men such as Hrdlicka, Wissler, Verrill, Lieutenant Bourke, Colonel Dodge and even Lewis and Clark in their journals have given a hearing to this important field of archeology which has made it possible for us of the profession to derive, concerning our more northern tribes an understanding of the matter which has been and still is of great value and considerable accuracy. May I also give some credit to the numerous amateur archeologists of our profession who have given time and study to the subject.

Considerable information must be also attributed to the most bitter enemies of Indian Medicine from the days of Cortez to the present time, the theologians who have with few exceptions mentioned but to condemn, such writers as the malodorous Cotton Mather for example. The father of this person, the Reverend Mr. Increase Mather also wrote extensively in like vein. Mathers *Magnalia*, Book III, as Halkett quaintly says, solves the Gordian knot of the matter of the origin of the American Indian most satisfactorily perhaps from the standpoint of a theologian. This reverend authority says: "The natives of the country had been forlorn and wretched heathen ever since their first herding here; and though we know not when or how the Indians first became inhabitants of this mighty continent, yet we may guess that probably the devil decoyed those miserable salvages hither, in hopes that the

gospel would never come here to destroy or disturb his absolute empire over them." The amiable Increase cites as illustrative of this archeologic gem, the statement of an Indian about to be executed for a murder committed while he was drunk, who stated that the "missionaries minded their bottle more than their Bible."

John Halkett, Esq., author of "*Historical Notes, Respecting the Indians of North America, with remarks on the attempts made to convert and civilize them*" (London, 1825), stresses the point that a natural approach to the affections of the Indians would be through the introduction of medical men instead of clergymen. This author points out the greater success of the Jesuits with the Indians because most of this sect were more or less medically trained, and they found the Indians very receptive to such teaching and quick to adopt better methods of medical treatment. The powerful influence wielded by men of the high character and attainments of La Salle and Hennepin, men whose lives and examples tallied with their preaching was thus fully explained, and very much of our most accurate and truthful statements in regard to the social and medical life of the Indians of North America will be found in the pages of the Jesuit Relations.

Franklin's essay on "The North American Savages" also gives us a very honest and faithful account of them. He says: "Savages we call them because their manners differ from ours, which we think the perfection of civility; they think the same of theirs." Unfortunately with the advent of the white man, smallpox, gonorrhea, scarlet fever, measles, tuberculosis, and other epidemic infections appeared for the first time among the Indians, who quite correctly attributed these scourges to the advent of the white missionaries and traders. Hence, it is that the universal antagonism of missionaries to the medicine men naturally developed, especially since the medicine men in all tribes have exerted a powerful influence over their people not only in matters of disease and medical practice, but also in general social and political life, in their theology; and in most tribes the medical men have been also leaders in war; in many instances either associate or chiefs during all times of hostilities. Many of our most famous Indians were of this class, Tecumseh, Sitting Bull, Chief Joseph, Geronimo, and many others.

In most tribes, especially with those of higher types of civilization, the medicine men were selected from the more outstanding, pious, and studious men of the tribe, sometimes, however, because they had passed through some remarkable experience or training. In other tribes, as among us, any person might assert his abilities and if he was able to develop a following he was allowed to do so, but in most instances the aspirant for medicine man was required to pass through a long and severe course of study or training under the auspices of some older and credited preceptor. In certain tribes the office was hereditary and young boys were put under strict training almost from their birth. In a good many instances likely boys were selected by the medical clan and trained by the older and more experienced men for their important office.

In most instances theology has been combined with the practice of medicine and the medicine men have attended or supervised the religious and sac-

ramental ceremonies as well as being charged with the care of the sick and injured. In some of the more highly evolved tribes the priestly and medical functions were, however, entirely disassociated. In almost all instances specialties were practiced; men especially skilled in the treatment of the sick were differentiated from those who attended to surgical disease, for example. In most tribes obstetric practice was usually limited to women, and many of the better tribes recognized both men and women as medical practitioners, in which case consultations and combined practice was usually in vogue. Women, however, rarely took part in war councils or in actual warfare while the medicine men almost without exception did, and as a general rule they were expected to act with particular valiancy and courage, because of their standing in the political, intellectual and governmental phases of tribal life.

My chief experiences with Indian practitioners as it happens, has been with the Ojibway people. For five consecutive years it was my happy privilege to spend a time each fall with the son of one of the most distinguished medicine women of this fine tribe, and through this relationship I was able to come into rather more intimate contact with the people than would otherwise have been the case. This tribe had, and to some extent still has, a most complete organization of its medicine men and women, for in this advanced people, women, properly trained and accredited were also admitted to the medicine clan, the Midi or Midiwin. Happily also the medical clan of this tribe has been most carefully studied by competent ethnologists and probably the medical organization of this tribe has been more completely studied and detailed than that of any other tribe in existence. (Read book of Diamond Jeness.) Two women of this tribe and clan, Mary Bear and Mrs. Steve L'Africaine, wife of a local Hudsons Bay Company chief factor, became quite friendly with me, and through them I met other members of the Mediwin Society. The deep respect in which I came to hold these people has inevitably tintured my attitude toward the Indian medicine man, a feeling but intensified by my less extensive experience with other tribes.

I shall attempt to give a garbled and very much abbreviated account of the organization of this society, largely gathered from reports and studies published by the American Bureau of Ethnology concerning the organization of the Midiwin.

The tenets of this society still permeate the Ojibway tribes and much of their medical practices are still derived from the society which is inextricably intertwined with the religion of this tribe.

Concerning these practices La Hontan (*New Voyages to North America*, London, 1703) says: "When they are sick they drink only broth, and eat sparingly and if they have the good luck to fall asleep, they think themselves cured. They assert that sleeping and sweating will cure the most stubborn diseases in the world. When they are ill they are visited by a sort of quacks, Jongleurs." Hennepin, in *A Continuation of the New Discovery*, speaks of these medicine men in a very resentful and critical manner. Marquette on the contrary, after referring to the Indian Herbs list and in mention of the calumet dance says: "They have Physicians among them, toward whom they are very

liberal when they are sick, thinking that the Operation of the Remedies they take is proportionate to the presents they make unto those who have prescribed them."

The symbol of the Fourth Degree of the Midiwin is the cross, and when Father Marquette found this he considered it as a compliment either to him or to his religion whereas it was but the symbol of one of the degrees of the Midiwin. In addition to the Midiwin these same Algonquin tribes have two other classes of priests or dealers in mystery, the Jessakkid and the Wabeno. These may or may not be members of the medical society, but their functions are quite distinct from those of the real practitioners who were members of the Midiwin almost without exception, except for certain general practitioners, as we would say of domestic or household medicine.

The Jessakkid is rated as a seer and prophet; the Wabeno is prompted by dreams and visions; he is a magician, indulges in exorcisms, etc. He may perhaps be rated in comparison to the regular practitioner of the Midiwin as the psychoanalyst is to us. The Jessakkid brings on evils and spells.

The existence of the society of the Midi is first cited by the Jesuit Relations about 1642. Probably the most understanding account of it, though confessedly very incomplete was published by an educated Ojibway, a member of the legislature of Minnesota, who also wrote the *History of the Ojibway Nation*. Warren states that even with his membership in the tribe, his perfect command of the language and his well recognized friendliness to his people, he was unable to secure absolutely authentic information as to the society, though Schoolcraft claimed to have been admitted as a member; if so he was probably the only white man who ever gained admission to the lodge; and Schoolcraft is quite generally thought to have been, to put it mildly, more picturesque than accurate in his statements.

Warren stated that the traditions and ceremonies of the society were not only transmitted by word of mouth but also by hieroglyphics and charts of ceremonies and procedures. These have of course now been largely lost or contaminated by admixture with the teachings of the missionaries of various sorts, by inattention and the lack of respect paid to the old men and their teachings by the younger generations.

The Rev. Peter Jones who was an educated Ojibway, serving as an Episcopal missionary, in his book (*History of the Ojibway Indians*, London, 1843) states that "each tribe has its medicine men and women, an order of priesthood consulted and employed in all times of sickness. These powwows are persons who are believed to have performed extraordinary cures, either by the applications of roots or by incantations. When an Indian wishes to be initiated into the order of the powwow, in the first place he pays a large fee to the faculty. He is then taken into the woods, where he is taught the names and virtues of the various useful plants; next he is instructed how to chant the medicine song and how to pray, which prayer is a vain repetition offered up to the Master of Life or to some munedoo whom the afflicted imagine they have offended."

Entrance to the society is obtained by a candidate applying to a member with whom he discusses the matter. If the application is favorably considered

it is then discussed with several members of the order, and in case of a favorable decision an instructor or preceptor is designated to whom he must present himself and make an agreement as to the conditions of his instruction, his fees, etc. The whole time of the student is at first not taken up and he has opportunity for hunting, fishing, and the maintenance of himself and his family. He may spend thus several years of study and preparation.

The society is graded into four degrees or periods of instruction.

The garbing of the practitioner differed according to the number of degrees which he had received in the society. Thus in the painting of the face, while acting officially:

First Degree: A broad stripe of green across the forehead and a narrow band of vermilion across the face, just below the eyes.

Second Degree: A narrow stripe of vermilion across the temples, the eyelids and the root of the nose, a short distance above which is a similar stripe of green, then another of vermilion, and above this again one of green.

Third Degree: Red and white spots are daubed all over the face, the spots of the size of the finger tips in applying the colors.

Fourth Degree: Two forms of decoration were permissible; the face was painted vermilion, with a stripe of green extending diagonally across it from the upper part of the left temporal region to the lower part of the right cheek; for the second, the face was painted red with two short, horizontal parallel bars of green across the forehead. Either of these was also employed as a sign of mourning by one whose son had been intended for the priesthood of the *Midiwin*.

The initiation of a qualified candidate into any of these orders was attended with very elaborate ceremonials and dignities, the recital of the history of the degree and clan. The ceremonies were usually if not always preceded by the ceremonial smoking of the pipe, and the Gods and progenitors of the degree were supposed to be invited and present usually in the form of some animal or totem believed to represent each deity or spirit. Initiation was usually celebrated during the latter part of the summer. Some at least of the ceremonies were performed in public so that the people might be duly impressed with their sacred, dignified and important nature. The main ceremonies were celebrated in a "Grand Medicine Lodge" built in an open grove or clearing, but the ritual was unintelligible to all save the initiated. The candidate was required to accurately memorize the ceremonial ritual and to transmit it in time to others, but charts were written, usually in picture writing on birch bark or skins, many of which have been preserved and studied by many students of the subject. These charts appear to me most difficult to make out or translate even with the key at hand, and of course many different interpretations have resulted.

Tobacco and other presents were furnished by the candidate, but charitable or devout members of the tribe, not participating directly were permitted to furnish gifts or contributions to the banquet and celebrations. The officiating priests were well remunerated. A specific preceptor or "docent"

was delegated to each candidate. The drums used in the ceremony were particularly constructed and consecrated. Naturally the occasions when so much highly charged "Good Medicine" was about was supposed to be particularly beneficial for the sick of the community who were expected to add of their wealth to the sacredness and dignity of the affair. Special rattles were used and the Migis, a small white shell of particularly mystical and sacred nature was extensively employed, particularly in the "Shooting of the Migis" in which the celebrant shot the spirit with the shell into the body and soul of the candidate, and the body of the sick attending the clinic. A certain amount of originality was evidently permitted the celebrants and the practitioner was allowed personal deviations as expressing his own peculiar power. The Migis shell was the sacred emblem of the society in general.

Midi Therapeutics.—During the considerable period of time in which the candidate was being instructed in the traditions, myths, songs, and ceremonies, the Midi plants were also discussed and the methods of identification, preparation, and administration of the therapeutic agents were elaborated. Specific agents and plants for definite complaints were discussed. The recognition of the complaints was necessarily mostly founded on symptoms, not on etiologic factors for little was known of this; for most, if not all diseases were believed to be of mysterious, divine, or devilish origin. In this regard it is highly probable that much of real value was further derived from the Catholic Fathers who for the time were fairly well versed in the medicine of the day; therapeutic effect was almost always attributed to the eviction of a bad spirit of some sort, and the efficiency of any method was supposed to be based on the superior knowledge or more powerful medicine of the celebrant. This did not, however, pertain to traumatic surgery in which the relation between cause and effect was fully recognized and acted upon; hence the surgery practiced was as a rule very intelligently based, and the treatments of dislocations, fractures, contusions and wounds was almost always very effectively carried out. They were skilled in massage, and in the application of active and passive movements.

Purification of the candidate was effected primarily by the sweat bath or ceremony, sometimes by the administration of cathartics and emetics. During these ceremonies the candidate was supposed to fix his mind on high and good things; he was fumigated by smoke, remained under certain taboos, as abstention from women and the like. Menstruating women were particularly supposed to injure the effectiveness and sanctity of any of these ceremonies.

In the conclusive stages mystical feats of legerdemain, sleight-of-hand, and the like to index the mystery and control which the medicine of the priests and candidates possessed. Many clever tricks were performed and just as among modern magicians the audience was not instructed as to the manner in which these seeming miracles were accomplished. Certain intimate ceremonies and instructions were carried out in private, in closed lodges and the like.

After the initiation proper had been accomplished the newly elected candidate proceeded to show his power and magic in shooting the Migis, produc-

ing syncope in his confreres, etc. The bear is a sort of tutelary spirit, the "Manido" of the society and enters largely into the mystical ceremonies.

Initiated practitioners might individually purchase from other members special songs, ceremonies, or methods which were supposed to be of particular merit; this was done outside the regular initiation ceremonies, but in the acquiring of a further degree he must gather together sufficient wealth and presents with which to endow the members of that degree. Thus certain practitioners may lead others to feel that he has a special medicine or mystery which enables the hunter to be particularly successful, to enable the lovelorn to bewitch his beloved and the like. In short, voodoo magic is quite well exemplified in many of the particular methods. Some of this "medicine" was doubtless of real value. Shrewd men of close observation of the habits of certain animals were able to impart for instance to the ambitious hunter information as to the taking of game, and the like which was the result of real keen study; most of it was probably just as valuable as the love spells and magic formulas which our young people still pay well for. A medicine man for example may from conversation with many hunters, from men of other tribes acquire a wide and valuable information as to hunting grounds, migration of animals, the methods of setting traps, and the like.

Many women took the degree of the Midiwin, but for the greater part their practice was restricted to women and children, but by no means always, if the modern practice which I have seen among the Ojibway is illustrative.

It is perhaps unnecessary to go into the details of initiation, into the various four degrees; it was largely a matter of the price and of the adeptness and personal character of the candidate. Initiation into the second degree cost twice as much as that to the first; the third degree three times; and the fourth and last degree four times as much. The "Grand Medicine Lodge" differed materially in each of the four degrees, just as it does in the conferring of the various degrees of our fraternal orders. Each degree had a chief priest, usually the senior, and on his death he was replaced by the election of a successor.

The amount of influence wielded by the Midi was general and immense, and particularly that of those who had taken the four degrees. They were believed to stand nearest to the great spirit and all the gods. A Midi Wabeno was also much dreaded because of his supposed supernatural powers.

The greatest just criticism which has been leveled against the Indian Medicine man has been because in most instances, except in traumatic surgery, which naturally played a very large part in Indian practice, of the mingled religious ceremonies with his practice; yet I have heard prayers for the sick offered up in nearly all churches and the various modern sects of our Christian faiths. Having witnessed both, I fail to find in the service of our modern faiths anything more dignified, beautiful, and worshipful than some of the chants or "dances" of the red man, conducted for the benefit of the sick, or for the purpose of imploring the assistance of their divine being in the welfare of their people. It must indeed be a very supercilious person who can fail to realize the sincerity and true devotional aspect manifested even today

in many of these ceremonies. The red man has never descended to the absurdities of Christian "Science," nor has he ever accepted anything more unscientific than the teachings of the osteopaths, chiropractors and the like, practices which are accepted by many of our wealthy, educated, and leading people. I do not believe that any flirtatious Indian girl was ever able to get away with such a story as that of Leda, of the cultured Greeks.

I feel then that we can well afford to ignore whatever of charlatanism, insincerity and faking which took place in association with Indian medical practice, since we ourselves are daily guilty of the same practices, in the universal seeking for the assistance of the mysterious, occult or religious, especially when we or ours are gravely ill. Time does not permit me to enter into this phase of medical practice or Shamanism, except to point out that our people of today practice exactly the same methods, though perhaps in a different form.

Suggestive therapeutics played a very important rôle in the practice of red medicine, just as most of us employ the same methods daily in our offices and hospitals. Many Indians were adept in the practice of hypnotism, and they employed this strange power exactly as we do today in our best psychiatric clinics. Certainly their understanding of this power was no less than that of the highly intelligent, scientifically trained Dr. Mesmer who was one of the greatest scientists of his time.

Time does not permit us to go into the matter of these ceremonies to any considerable extent, but we cannot leave this phase of our subject without reference to the Medicine Lodge and the important rôle which it plays both as a devotional observation and as a method of medical practice in practically all tribes.

The Medicine Lodge was a term commonly applied to a structure erected strictly according to many solemn ceremonies, designed to propitiate an angered or careless God or to express the spirit of humiliation, thanksgiving, or supplication, either on the part of a person or a people.

It was constructed on occasions of momentous national affairs when the whole tribe or tribes were gathered together for the purpose of council, because of some great public or personal calamity, epidemic sickness, or because of some personal complaint of so grave a nature that it was not felt that ordinary or usual priestly or medical ministration was sufficiently weighty. Even when the lodge had been built and the people and its priests gathered for the purpose of discussing some tribal or national matter, as the advisability of going to war for example, it was felt that the moment and the place was propitious for the treatment of any private physical ill.

Persons of great piety, wealth, or who, because of some personal blessing, illness, or misfortune or vow, wished to undergo the necessary expense attendant upon the building of a medicine lodge might undertake it, of course under the guidance and instruction of the chief medicine man.

Having been constructed according to the ritual, certain ceremonies, depending on the purpose of the lodge, were then performed by the medicine men after which the real object of the ceremony was reached, whether it was a council of war or for the treatment of some sick person.

Almost invariably, because of the universal employment of the sweat, either in religious or for purely medical purposes, the building of the medicine lodge was accompanied or preceded by the building of a sweat lodge, sometimes a large structure accommodating several celebrants, or a smaller one designed for but a single person at a time. At times the ceremony of sweat seems to have been carried on in the large structure, that is, in the real medicine lodge, but it appears to have been more usual that those who had been first prepared by the sweat were admitted to the ceremonials in the great lodge.

As an example of the type of chant or litany which was employed in the course of the treatment of diseases, I wish to quote the Sacred medicine formula of the Cherokees, employed by Gahuni, a famous medicine man of that tribe, in rheumatism known as "The Crippler." Gahuni died about 1855. I shall allow you to judge if it is more foolish than the mumbo of the successful quacks of today. (Seventh Annual Report, Bureau of Ethnology, 1885-86.)

"Yu! O Red Woman, you have caused it. You have put the intruder under him. Ha! Now you have come from the Sun Land. You have brought the small red seats, with your feet resting upon them. Ha! Now they have swiftly moved away from you. Relief is accomplished. Let it not be for one night alone. Let the relief come at once."

"Repeat this prayer four times. (If a woman you must say 'Red Man' and if a man, you must say 'Red Woman.') Meanwhile laying on hands, take off the hands; blow once at the fourth repetition, blow four times. And this is the medicine. Egluni (a fern), Yat na-Utsesta (Christmas fern), Ka ga Asgutage (Maiden hair fern), Da yi-Unwayi (Beavers paw). Boil the roots of six varieties together and apply the hands warm with the medicine upon them. Doctor in the evening. Doctor four consecutive nights. (The pay) is cloth and moccasins, or if he does not have them, just a little dressed deer skin and some cloth."

And this is the tabu for seven nights. "One must not touch a squirrel, a dog, a cat, mountain trout, or women. If one is treating a married man, he must not touch his wife for four nights. And he must sit on a seat by himself for four nights, and must not sit on the other seats for four nights."

Religion is always a difficult subject to discuss dispassionately. The Indian had his religion, and whatever his beliefs, he took it quite as seriously and much more faithfully than most of us do. My own private belief is that he is quite as much entitled to his beliefs as we are to ours. Whatever of brutality he may have shown has been quite equalled by many of those who profess the faith of the gentle Jew of Nazareth. As in the early days of our own medicine, religion has also been intimately mixed with the medical practices of the Indian, and this is the chief criticism which we may level against it. Psychotherapy, however, is a very important agency for cure in many, if not most diseases, and the red man has realized this perhaps more fully than we of this age do. As has been recently said by one of our leaders (Thomas Salmon), "No medical system can be complete and fully satisfactory until the proper commingling of psychotherapy with the methods of real science of pathology, chemistry, and bacteriology has been found." Until we medicine men of today have properly realized and adopted this fact, we are in no position to criticize the red prac-

titioner who did without question practice this associated psychotherapy and physical practice with notable success and well adapted to the requirements of his patients.

Naturally for an intensely religious people, the theory of disease among practically all tribes is the same, namely that of an evil spirit or bad medicine which has entered the body and caused sickness. Obviously the thing to do in that case is to drive out this evil spirit by a stronger good medicine, for which quite naturally the gods must be appealed to through proper prayers and medicines. Hence, it is that most of the incantations practiced or in vogue are such as the shooting of the Migis of the Ojibway and the sucking out of the disease which is so generally practiced not only by Indians but also by simple whites and even by animals. As is well known the medicine man in such treatments is likely to conceal in his mouth or elsewhere on his person a stone, or perhaps some living object as a cricket, frog, or the like which he dramatically produces at the proper time.

I have known of many whites who also believe in the efficacy of prayer and of divine intervention in cases of sickness. Perhaps we all are somewhat inclined in this direction to a greater or lesser degree, so why laugh at the Indian? Our church services seem quite as absurd to him, so I am told, as his ceremonies do to us.

Of course the shaman performance opens the ceremonies of the Indian to the ridicule which the superior white is likely to visit upon it, and the whole thing is then considered as a bit of charlatanism. I do not, however, agree with this conception for I am convinced that in many instances at least, the removal of the foreign body on the part of the priest has precisely the same significance as the very similar ceremonies practiced by our shamens. The attempt to deceive and willful faking is not in the mind of the respectable practitioner any more than it is in the mind of our psychiatrists when they do practically the same thing in their practice. Those of you who have seen these treatments given, cannot I am sure, have failed to note the reverential attitude with which it is usually conducted. The psychic benefit to the patient at least is real.

It is, however, a real pleasure to turn from these phases of medical practice to the methods of the Indian which commands our respect as scientists and as modern physicians.

Long before the days of Hippocrates, of Paracelsus, long before Koch, Ehrlich and Pasteur, the Indian medicine man practiced an art which came close to a science which even today commands our utmost respect and emulation.

Before Egypt was a nation, long, long before the dominance of the Greek school, Verrill with others, has shown us that the Indian medicine man practiced successfully Cesarean section, symphysiotomy; he understood and applied the wide use of trephining; he unquestionably used cocaine as an anesthetic together with other effective sedatives and analgesics; he installed dental plates and bridgework; he even supplied his patients with artificial limbs.

The writings of practically all our army surgeons shows that even the Indians of the days of Custer, Crook, and Miles well understood the treatment of fractures, dislocations, of stab and arrow wounds, and his treatment of gun-

shot wounds in the more intelligent tribes was efficient and in many respects admirable. I myself have seen something of this surgical practice. I have seen the most clever sort of splinting in fractures; I have seen ball and stab wounds well treated, the lips of bulging or gaping wounds cleverly approximated, and others have reported the use of thorns and skewers for this purpose. Shrewd and hard-headed men such as Alaes Hrdlieka, himself a skilled physician as well as one of our most eminent ethnologists and anthropologists, has spoken frequently in unstinted praise of some of these methods of surgical treatment. I have also observed as a youth much of the efficiency of pioneer medicine which owed its origin mostly to the red medicine man.

I have heard my own parents speak in terms of affection and respect of the work of Old Baptise, medicine man of the Sioux whose services were so respected and valued by the white settlers of Minnesota that when his tribe was sent to a reservation, he was asked to remain, supported by the government because of his value to the early settlements of Minnesota. I cannot say that many of the doses of "simples" which I received as a child were loved at the time by me but at least we grew up to be as healthy, sane and sound as the men and women of more advanced communities. Whatever may be said against Indian Medicine, it was also that of the pioneer in very large degree. The reports of army officers, particularly of medical officers is almost universally respectful of some at least, of the medical customs of the Indian. Furthermore almost without exception explorers, miners, settlers, and those others who have lived among the Indians have universally cited frequent instances in which strikingly successful treatments have been given by these primitive practitioners. I am certain that few of us who were born of pioneer stock or who have lived among even the modern tribes hold anything but respect for what we have seen and experienced of Red Medicine. Verrill cites, for example, his successful and comfortable recovery from yellow fever under the care of medicine men.

Let us then consider in some detail certain of the drug uses and other practices for which we are entirely dependent upon the Indian. I shall pass over these drugs very briefly, for I am confident that most of you know the more important of them. Perhaps we should put at the head of this notable list, quinine because of the great rôle which it has played not only in individual medical practice, but also because without quinine the march of civilization, great engineering and military projects must have been halted or become entirely impossible. Cocoa, with its derivative cocaine which has become so universally employed in many branches of medicine, we also owe entirely to the South American Indian. Cascara is also of Indian origin. There are many others peculiar to Indian origin, but usually in less frequent general recognition by our present-day profession. As for drugs which are universally employed, and the direct origin of which no man knows, they are very numerous. Saline laxatives were widely employed in practically all districts where nature supplied them; we might say the same for most of the wild animals also, as every field hunter knows. Foxglove, a crude form of digitalis, was widely employed in the districts where it grows. The list of laxative drugs used by the Indian is very large, and from personal experience, I can definitely say

that they are highly effective. Diuretics also are very numerous and highly efficient; mostly they are decoctions of various plants, and commonly they are administered in warm or hot water, which of course greatly increases their effect.

Emetics, now rather infrequently employed in our practice were and still are very extensively used by the Indians. Under many circumstances they were employed for religious purposes, as for example in the course of the preparation of the celebrants of the snake dance, but they are also very generally used in any manner of gastrointestinal disturbance much as we use laxatives in very many disorders, empirically. There is a tremendously large list of drugs used for the purpose of emetecesis, including large quantities of warm water and irritation of the fauces, after our own manner. Decoctions in warm water of holly, cucumber, thoroughwort, blood root, etc., ad infinitum. Among the Navaho the fermented urine of cayuses is used, and I am informed by an Indian trader, quite familiar with the Snake Dance ceremony, that the fermented urine of the burro is usually employed in this ceremony, supposedly because it has also some antitoxic effect against the bite of the rattlesnake.

I have found it extremely difficult to get accurate information from my Indian friends, even when they were entirely convinced of my sincerity. They are as a class very sensitive, fearful of ridicule, and most evasive in their answers to questions. I have found my most successful avenue of approach to speak as a member of the same general medical fraternity, seeking from them further information, usually telling them what I did in certain cases and asking in turn what they would do under similar circumstances. Occasionally I have been able to establish thereby a quite satisfactory relationship, but I am fully convinced that most intelligent medicine men also employ a good many valuable drugs of which we know nothing, and I am certain that scientific study of some of their preparations would be of great benefit to us. Many explorers and ethnologists have made this statement to me also.

It is quite natural that the Indian should be skilled in meeting particularly the conditions which he contacts in his ordinary life. This is shown especially in his management of snake bite. There are of course several varieties of snakes whose bite is invariably, or practically always, fatal. Against some of these reptiles they have no antidote, but I am informed by field ethnologists, explorers, miners, and the like that they are certainly able to successfully treat the bites of many supposedly highly virulent forms of snake bite. Not infrequently whites exposed to this peril have profited by this knowledge, but I have never known an Indian to divulge this secret.

The trader at Oribe who had been among the Pueblo people many years told me that he had never seen a case of fatal snake bite among those tribes to whom the snake is sacred, and yet who handle them frequently and are often bitten by them, ill-protected as they are by scanty clothing. In the famous snake dance, this same man told me that he had never known serious illness to follow the bites incurred in this ceremony although bites from the western rattler are very frequent in the chant. Of course this has been in part accounted for by the fact that for several days preceding the dance the snakes are very much disturbed and the probability is that their poison is largely exhausted by the

time of the actual ceremony. Still it is certain that of the several preparations taken before the dance, in the purification ceremonies, that antidotes are taken, whether with success or not cannot be determined. During the emetemsis which precedes the dance, the urine of the burro is taken in large quantities because of its emetic effect, and it is supposed by some that this acts also as a protective. They very generally in case of bites from poisonous reptiles, practice as we do, scarification and local blood letting, and this doubtless helps some, but face, neck, and arm bites are common during the dance. At least the dancer goes on with his ceremony with apparent indifference. The Indian bystanders, not participants, are certainly quite as adverse to the snakes as we are. I think that we must conclude that they do have protective measures of which we know nothing.

Febrifuges are in common use. dogwood for example, which contains cinchona in considerable quantities is so used in decoctions. My Yanqui guides in Durango, Mexico, used infusions of sarsaparilla among other preparations for this purpose, and I know from personal experience that they are effective in considerable degree.

Astringent decoctions, abstention from food, and rest are widely and successfully used in diarrhea. My Ojibway friends used a decoction of spruce or oak root for this purpose. I think each tribe has useful preparations for this purpose, depending, of course, on the plants or drugs available. My mother used fermented decoctions of wild blackberry, for example, for this purpose, and I recall that when I went away to study medicine my mother provided me with a liberal supply of this, which she had, as a pioneer woman, learned to use. It was not only effective but also very pleasant, particularly when it had sufficiently fermented. On several trips my Indian guides have supplied us with very effective preparations of like effect; but it is exceedingly difficult to get them to tell what these preparations were, notwithstanding the fact that they were certainly most kindly inclined toward me.

Very much has been said as to the immunity of Indian women from the ills peculiar to our women, of their quick and easy labors, immunity from perineal tears and the like. So far as the recent Indian is concerned, I think that these statements are incorrectly based. It is true that most Indian women do get about immediately after labor; it is also true that they very frequently have very severe obstetric difficulties as do our women. It is very true that they are stoical and indifferent to pain, for that is the custom of the people who are not given to emotional demonstrations of this kind, but from my conversations with agency doctors and from my contacts with the Indians, I am certain that they are quite as liable to difficulties of this kind as the same social types among white women, that is as compared with pioneer women, farm women and the like, but of course not comparable with women who are soft of muscle, overly fastidious, and the like. I have seen plenty of Indian women who suffered extremely from dysmenorrhea, menorrhagia, and similar conditions. I do think from my own observation and from the statements of reservation physicians that fibroma and myomas are much less frequent among them than with us. I well remember overtaking a party of Ojibway friends encamped on their way to the hunting fields because they did not wish to leave behind a favorite daughter of the chief,

who was suffering from dysmenorrhea and menorrhagia. They were as anxious as I was to get on to their hunt, but the chief told me that women were a great hindrance to tribal activities because of the troubles incident to their sex.

Malignant growths are certainly very much less frequent among the Indians than among whites. In fact they are rare among the unmixed tribes. I have never seen a malignant growth in a pure blood Indian, and reservation doctors report to me the same; it is also remarked upon by Hrdlieka. I looked into this matter last year while in Mexico, where the population is preponderately Indian. In many places tribes, like the Yaqui, have preserved to a marked degree their racial purity. Every physician with whom I came in contact made the same statement that malignant growths were exceedingly rare among the Mexican Indians. Dr. Palomecque, a young and exceedingly brilliant surgeon, had practiced two years in the city without seeing a single instance of gastrointestinal carcinoma in an Indian, though he had a large public charity practice. Dr. Ramon Molina, probably the leading internist of Mexico City also confirmed the same statement; and among the other physicians, including a leading pathologist, the same opinion was voiced. Hence it is that there appears to be very little knowledge among the Indians concerning newgrowths. They are certainly very rare, as is also true among pure strains of wild animals. Mr. Fenley Hunter of the Explorers Club has recently reported to me the death of a well-known squaw, Big Mary of carcinoma at Fort Yukon. The diagnosis was conformed by Dr. Hrdlieka.

The same appears to be true of thyroid disease, though I have seen simple nontoxic goiter in Indians living in the so-called thyroid districts.

Naturally rheumatism is one of the most serious diseases with which the northern tribes have to contend, the Ojibways, the Crees, and the northern tribes in general. I believe that it is rarely seen in the south, among the Pueblos, the Navahos, the Mayas, and so on. Naturally in those peoples greatly afflicted with this disease with its high disabling effect, treatments are very numerous. Perhaps in this respect they practice polypharmacy as liberally as we do in the same condition. Hot applications, the inevitable sweat bath, demulcent drinks and many decoctions are widely employed, and certain tribes use willow bark and twigs in the form of a decoction. Among the Midiwin practitioners the willow ash, which is, of course, high in the salicylates is extensively employed. I was shown its preparation by a very fine old gentleman, one of the Midiwin society of the Ojibway. This man was not only highly intelligent, but a real gentleman, if acts outweigh clothing; and he was also a most artistic and ingenious builder of the birch bark canoe, now practically obsolete. Massage, active and passive movements were employed extensively both in this disease and also in the treatment of sprains, bruises, and so on.

Anodynes and anesthetics were doubtless more extensively used and elaborated by the South American tribes, the Pre-Incas and Incas, Colimas, etc. It must always be remembered that in these sections, particularly about Lake Titicaca, an order of civilization existed which beyond doubt exceeded that of any other human race at the time of its zenith, and in many respects this persisted, though in a degenerated type, up to the time of the Spanish conquests. But our story has chiefly to do with the Indians of the times of our forefathers.

Stramonium of several kinds was used rather extensively both to quiet pain and also to produce a condition of semiintoxication in certain ceremonies. Even up to recently the government has attempted to prohibit the use of the peyote, which induced a condition of intoxication, comparable to the use of opium. Aleoholies were and still are in extensive use among the Indians of Mexico, in the form of mescal, and the favorite present-day drink in Mexico is pulque. Both these drinks, unless most artfully disguised, taste most terribly to the unaccustomed palate, but the last mentioned forms a considerable article of the diet of the ordinary Mexican. The northern tribes as a class used aleoholies very little. These preparations were very extensively employed by the tribes familiar with them, in various nervous conditions, for mental and nervous diseases were familiar enough in Indian Society. I have seen as characteristic and striking hysterical attacks among young Indians as one might wish for. As I have previously mentioned, among the members of the Midiwin, the shamans who undertook the treatment of neurologic diseases were rated in the last or highest chapter of their "Grand Medical Society." As has been noted by very many writers, mental disease was rated among the plains tribes in general as being related to the Great Spirit. These sufferers were judged as to a considerable extent irresponsible, or under the special care of the Great Mystery; hence they were treated with great kindness and consideration, a fact which was utilized, we are told, with advantage by prisoners falling into the hands of these tribes. At least we must consent that such a method of management was considerably in advance of the treatments for the insane, prevalent almost up to the time of Chareot among some Christian peoples. Our amiable ancestors of New Bedford and Salem might have taken a beneficial lesson from the red barbarians.

Treatment of contagious diseases was very badly carried out as a rule, and it is probable that in most cases it was harmful rather than otherwise, since the sweat lodge and cold plunge apparently represented the most prevalent method. It must, however, be remembered that apparently most of the epidemic contagions were introduced by the white man, typhoid fever, smallpox, measles, scarlet fever, diphtheria, and the like. Probably the less said about these matters, the better. It is quite certain, as I have said, that nearly all of these contagions whether treated by medicine men or agency physicians are terribly fatal; whole tribes within my memory have been almost wiped out by measles, for example. Darwin mentions this fact in his *Voyage of the Beagle* and even at that early day, he made a correct interpretation of its significance; for he remembered that this great scientist had been a medical student, and that he never lost his interest in medical science.

Venereal diseases were very prevalent following the early contacts with the whites. They were for the greater part very poorly treated. This was the result of overly generous social customs, frequently mentioned for example in the voyage of Lewis and Clark. It has been told me by several well-qualified men that the southern tribes in Mexico have a plant, the Yerba Santa which is effective in syphilis. There is no doubt, I think, but that the Indian has now acquired an immunity against syphilis which is higher than that of most whites, but syphilis acquired from such infected tribes is likely to run a very malignant

course when acquired by the whites. Hrdlicka asserts with authority that there is no evidence of pre-Columbian syphilis, though the disease syphilis was spoken of variously as the "Mandan Disease," the "Gros Ventre Disease," and so on from its supposed origin from the Indian.

The subject on which I have ventured to speak to you is a most interesting one; it has pleasantly occupied much of my spare time, and has given an added interest to my trips into the wilds ever since my boyhood days. I am certain that future study of the medical customs, especially of the South American tribes and of certain of their drugs will certainly add to our armamentarium other drugs, perhaps as valuable as quinine and cocaine. The late Dr. Rusby, known to all of us for his researches along these lines frequently made this statement. Doubtless when the archeologists have found the key to the inscriptions of the Tiahunacans, the Preineas and the Incas, the Mayas and Toltees, we shall find further evidence on medical matters of great value to the world, just as artists, sculptors, architects, economists, and students of government await anxiously positive accounts of the wonderful people who preceded us on this continent.

We may at least be certain that the medical practitioners of these people have played an important and honorable rôle in the early history of America; one which reflects honor on us who have followed them in the study and practice of the healing art. It is certainly regrettable that we have long been so blind to the great part which the prehistoric physician of America has played, and that we are still so prone to feel that in the history of progress in art, architecture, mathematics, astronomy, and even in government, we owe most to the old world while the sciences of archeology and ethnology have definitely now shown to be not in advance, but behind the early civilization of the Americas.

In my talks on this fascinating subject, I have been frequently asked for references to literature on the subject. In the hope that you may find as much pleasure and benefit in the study of this subject as I have, may I refer you first to the numerous publications of the Department of Ethnology. Get as many of the old volumes as you are fortunate enough to be able to procure. Have at hand the Encyclopedia of the American Indian. It is now old, and does not include recent discoveries, but it is most interesting none the less.* Read Schoolcraft, though he was a picturesque liar; read and study the egotistic inaccurate but fascinating and courageous story of Catlin; his plates are none the less our first artistic data on the life of the American Indian of the days of our grandfathers. Read and reread Lewis and Clark, particularly the edition of Dr. Elliot Cowes. Read Colden's *Indians*, Colonel Dodge's *The American Indian*. Read the early publications of Lieutenant Bourke. Do not forget the valuable material to be found in the Jesuit Relations, the tale of Father DeSmet; read and marvel at the story of the courageous Cabeza De Vaca, of Coronado, of Pizarro—and when your palate tires of detective stories, read the diary of Bernald Diez del Castillo, the Lieutenant of Cortez. Do not forget Prescott's *Conquest of Mexico*, or his charming story of the *Conquest of Peru*.

Read the carefully compiled volume on *Shamanism* of Dr. Maddox. Gather to your choicest volumes everything original that you can find on early American history, and the records of the colonists both of our own United States and of

*A new edition is now under way.

Canada. Read Franklin's *Essay on the American Indian*; Cotton Mather. Read Schultz's *My Life as an Indian*; read every book that you can lay your hands on written by Verrill; I believe that there are only seventy-nine volumes as yet. Read the publications of Dr. Alaes Hrdlicka, the charming volume of Dr. Hough *The Hopi*. Everything written by Clark Wissler is beautifully done and most authoritative. All the publications of the American Museum on the subject are well worthy of their origin. There is a very fine little volume, recently written by Dr. Erie Stone in the series, *Clio Medica*, on *Medicine Among the American Indian*. Better still visit and revisit the marvelous collections of the Museum of the American Indian, Heye Foundation, the American Museum of Natural History, the choice though small, and local collection in the City Museum of Milwaukee; and you will soon find yourself equipped with a most fascinating hobby which will bring you pleasure and profit just as long as your cerebral cortex is intact. Your library will grow by leaps and bounds; you will haunt the old book stalls, and your spare change will not go to Wall Street.

Best of all, visit the Mesa Verde, Pueblo Bonito, Kitseal, Betata Kin, the still active Indian villages of Zuni, Oribi, Walpi, and the pueblo towns in general of New Mexico and of Old Mexico. Marvel in Mitla, Mont Alban, the City of Mexico with its unequalled collections and its dramatic history and startling views. And there is still Yucatan, with its preposterous and beautiful old cities, older than Egypt. There still remain for you, all of Central and South America with Cusco, Tia Huanaca, and the old Preincan and Incan towns of Peru, Bolivia, Chili—then you have started on a lifetime search of the beautiful and marvelous, on a study of the history of the country where "Columbus Came Late," but where you and I live, in a land with a history and heritage elsewhere unequalled.

FUNCTIONAL STUDIES OF PATIENTS ON ANTIARTHRITIC MEDICATION

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THE primary purpose of this study was to observe the functional reaction of unselected arthritics to a few standard types of medication. Accessory modes of treatment which were based upon the etiology, and aimed at curative effect such as the elimination of foci of infection, were carried out in all cases. From the standpoint of this study, however, the latter methods were of academic interest only. The secondary purpose of this study was to observe whether, in a comparatively small series (143 cases) of arthritis, under moderate dosage of common drugs, idiosyncrasy or lack of tolerance by the kidneys or liver was frequent or the reverse.

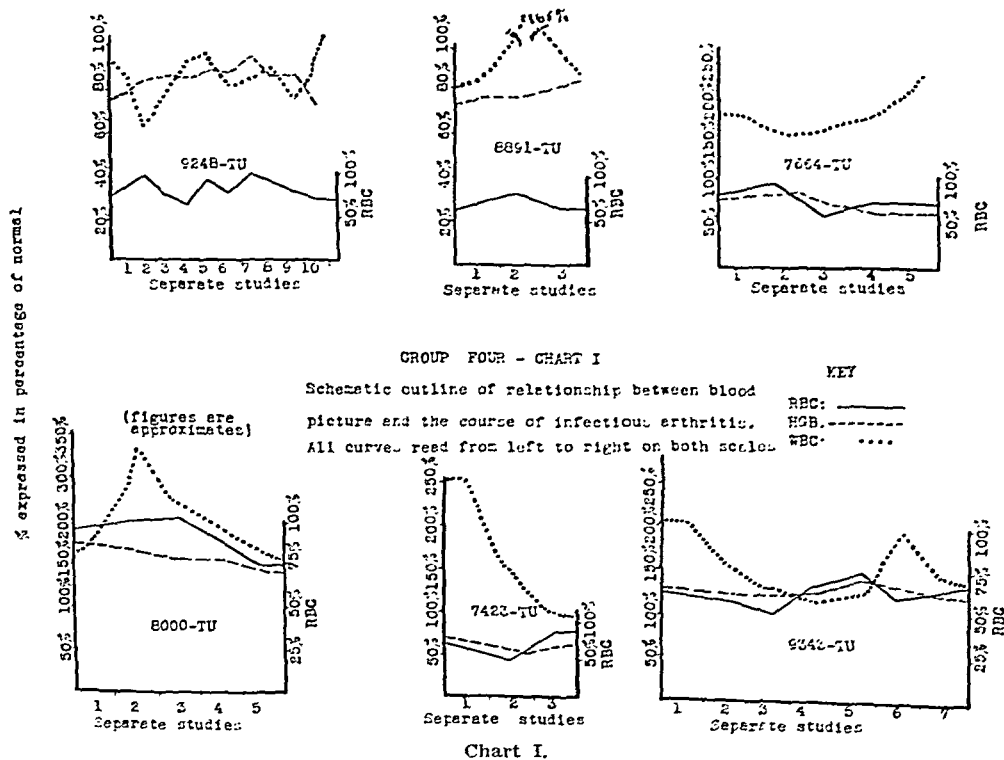
In 1923, Worster-Drought¹ published one of the first alleged cases of phenyleinchoninic toxicity, which recovered. In somewhat desultory fashion other cases, with recovery, were reported for the subsequent three years. In 1926, Langdon-Brown² published one of the first³ reports of a death, due to atophan. From that time to the present, there have been reported several cases of intoxication due to phenyleinchoninates. The authors appear to have overlooked the fact that not only does acute yellow atrophy arise from many causes and many drugs, but that the well-known salicylates have also been known to cause this condition.^{3, 4, 5} Further, authors appear to have failed to compare the annual consumption of phenyleinchoninates and salicylates with the relative frequency of symptoms of intolerance. Since the manufacture of the former runs to almost one hundred thousand pounds, and of the latter to three million pounds† in the United States alone, it must be fairly obvious that the chance of intoxication, statistically, is slight.

There is a large bibliography on these questions of toxicity which it is scarcely necessary to cite. It seems to us that further work in the clinic, coupled with some of the newer laboratory studies, should be done to amplify the findings of the earlier writers such as Hanzlik.²² As to the use of phenyleinchoninic acid (cinchophen) itself in these studies, we were of the opinion that the position taken by the *Journal of the American Medical Association* in 1931 was sound; and that we would, accordingly, not use phenyleinchoninic

*We consider the case reported by Cabot (Boston M. & S. J. 192: 1122, 1925) to be erroneous and so shown by the report in the J. A. M. A. 89: 1167, 1929.

†U. S. Census of Dyes and Synthetic Organic Chemicals: U. S. Tariff Commission, 1930, pp. 60, 61. Manufactured Salicylic acid 2,781,196 pounds, Cinchophen 93,765 pounds.

acid. The statement said (J. A. M. A. 97: 409, 1931): "... it would be well to discontinue the use of cinchophen, or atophan, and to substitute neocinchophen for it. . . . This remarkable safety of neocinchophen is also reflected by the fact that it is practically impossible to find one undoubted case of neocinchophen liver atrophy in the literature." These facts, then, that neocinchophen and the salicylate of sodium have been so very rarely accused, led us to use these drugs in our study. We are in agreement, so far as the clinical side of the picture is concerned, with the conclusions of Davis,⁷ who has recently said: "A study of 200 cases taking neocinchophen or cinchophen has been made. . . . The superiority of neocinchophen to cinchophen for general



clinical use appears, at the present time, a fair conclusion from the evidence of the literature and the author's cases."

In the matter of nomenclature, we have been guided by the *Rheumatism Primer* published by the American Committee for the Control of Rheumatism. As they observe, no classification suits everyone or every case, but a simplified classification aids us in the comparative judgment of reported cases and hence should be adopted where possible.

Our laboratory tests² have been the classical ones as described by Hawk and Bergeim,⁸ Meulengracht (Brown's modification),^{9, 10} van den Bergh,¹¹

²To avoid prolixity we use the quite widely recognized abbreviations of E.K.G. for electrocardiogram; B.S.P. for the bromsulphalein test; and P.S.P. for the phenolsulphonphthalein test.

Bauer,¹² Wallace and Diamond,¹³ Rosenthal and White,¹⁴ Cantarow,¹⁵ Davies,¹⁶ and Rowntree and Geraghty.¹⁷

Blood pressure studies were made on every patient; and on some patients as many as twenty or thirty determinations were made, but we found nothing of the slightest functional significance. Many blood picture studies were made, and the results in a few cases, as regards progress of the infection and secondary anemia, have been shown in Group 4 chart.

The rationale of our procedure is more clear if we consider the cases in an order inverse to the complexity of the findings. One of us (W. C. M.), seeing constantly, in his office, a considerable number of industrial cases, observed clinically, without laboratory study, 70 unselected cases of traumatic arthritis upon minimum medication of one type. The other of us (D. S.) observed similarly, at the various dispensaries to which he was attached and in his office, 50 unselected cases of arthritis, usually infectious, upon another type of medication, in minimum dosage. The first 70 case series was composed as follows:

Arthritis of the:

Feet	8 cases or 11 %
Knee	11 cases or 15½%
Ankle	5 cases or 7 %
Lumbodorsal vertebrae	5 cases or 7 %
Digits	5 cases or 7 %
Pelvis, including the sacroiliac juncture	6 cases or 8½%
Forearm, including the wrist	7 cases or 10 %
Upper arm and shoulders	4 cases or 6½%
Miscellaneous traumata about the muscles, femur, ribs, legs, etc.	19 cases or 27½%

The age distribution of these cases was as follows:

10 to 20 years	1 case
21 to 30 years	15 cases
31 to 40 years	27 cases
41 to 50 years	20 cases
51 to 60 years	6 cases
61 to 70 years	1 case

The sex distribution was: males, 67; females, 3. The youngest patient was eighteen years of age; the eldest, sixty-seven. The average length of treatment was four weeks; the shortest, one week; and the longest, twelve weeks. Every patient received 5 gr. of tolysin* three times a day. The symptomatology was affected as follows:

Moderate relief	19 cases or 27%
Marked relief	49 cases or 70%
No relief	2 cases or 3%

*A brand of paramethylphenylcinchoninic ethyl ester, manufactured by the Calco Chemical Company, Inc.; other names being used in the literature for this chemical are neocinchophen, novatophan, etc.

While laboratory studies were not done upon these patients, none at any time complained of gastrointestinal, hepatic, or renal symptoms, or showed the signs of such organic dysfunction.

In a manner roughly parallel to the above method, 50 ambulatory cases were observed by one of us (D. S.) under minimum medication with elixir sodium salicylate compound (N. F.). They received the equivalent of 5 gr. of sodium salicylate plus potassium iodide, gelsemium, cimicifuga, etc., in an alcoholic medium, three times a day. These unselected office and dispensary cases of arthritis may be anatomically subdivided as follows:

Arthritis of the:

Ankle	7 cases or 14%
Wrist and hand	6 cases or 12%
Elbow	1 case or 2%
Shoulder	6 cases or 12%
Knee	7 cases or 14%
Hip	1 case or 2%
Sacroiliac joints	3 cases or 6%
Several joints	10 cases or 38%

The ages were distributed as follows:

10 to 20 years	3 cases
21 to 30 years	4 cases
31 to 40 years	24 cases
41 to 50 years	8 cases
51 to 60 years	8 cases
61 to 70 years	2 cases
Unknown	1 case

The diagnostic classification was as follows:

Atrophic Arthritis	43 cases or 86%
Hypertrophic Arthritis	7 cases or 14%

The youngest patient was nineteen, the eldest, sixty-nine years. The sex distribution was: 38 female, 12 male. The length of treatment varied from about one week to about eight weeks, on an average four weeks.

The results obtained were:

Moderate relief	44 cases or 88%
Marked relief	3 cases or 6%
No relief	3 cases or 6%

No symptoms or signs were observed traceable to renal, gastrointestinal or hepatic dysfunction.

The above two series are obviously antithetic as regards etiology, sex, and, to a certain extent, the distribution. Nevertheless, together they cover nearly the whole range of ambulatory arthritis. In general, the symptom to be re-

TABLE I
GROUP ONE—TABLE OF CLINICAL TESTS ON THIRTEEN HOSPITALIZED UNSELECTED CASES OF ARTHRITIS

DIAGNOSIS	9218-TU HYPERTHORIC ARTHRITIS		6676-TU ATHROPHIC ARTHRITIS		10726-TU ATHROPHIC ARTHRITIS		7168-TU ATHROPHIC ARTHRITIS		8533-TU ATHROPHIC ARTHRITIS	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
URINALYSIS	v. ft. tr. albumin, no casts	sl. tr. alb. no casts	none	ft. tr. alb. Occas. hyaline cast	no alb. no casts	v. ft. tr. alb. no casts	tr. alb. no casts	tr. alb. no casts	tr. alb. no casts	ft. tr. alb. no casts
P. S. P.	1 hr. 20% 2 hr. 30% 3 hr. 10%	1 hr. 25% 2 hr. 20% 3 hr. 11%	none	33% 24% 59% q.n.s.	none	25% 20% 15%	1 hr. 10% 2 hr. 27% 3 hr. 32%	1 hr. 5% 2 hr. 13% 3 hr. 7%	8% 10% 25% 7%	
B. S. P.	none	5 min. 5.0% 30 min. 0% 60 min. 0% normal	none	0% 0% 0% normal	none	35% 0% 0% normal	5 min. q.n.s. 30 min. 0 60 min. 0	none	25% 0% 0% normal	
Van Den Bergh	Direct: neg. Indirect: <0.2 mgs.	Direct: neg. Indirect: <0.5 mgs.	none	Direct: neg. Indirect: <0.5 mgs.	none	Direct: neg. Indirect: <0.2 mgs.	none	Direct: neg. Indirect: <0.2 mgs.	none	
Icterus Index	7.0	8.0 (avg.)	none	1.0	none	9.0	3.0	5.0	none	
Wassermann	negative	none	negative	none	negative	none	negative	anticomplementary	none	
Kahn Reaction	negative	none	negative	none	negative	none	negative	negative	none	
Blood Chemistry	Urea-N. 15.5	Urea-N.: 15.5 Uric Ac.: 4.1 Creat.: 1.1 Dextrose: 129.8	none	none	none	none	Urea-N.: 18.0 Uric Ac.: 3.7 Creat.: 1.1 Dextrose: 110.0	Urea-N.: 34.0 Uric Ac.: 3.8 Creat.: 2.1 Dextrose: 85.0	none	
Galactose Test	none	1.5 gms. /5 hr.	none	2.3 gms. /5 hr.	none	2.3 gms. /5 hr.	1.5 gms. /5 hr.	1.5 gms. /5 hr.	0.135 gms. /5 hr.	
Urobilinogen	none	1:10 neg. 1:20 neg.	none	1:10 pos. 1:20 neg.	none	1:10 pos. 1:20 neg.	1:10 pos. 1:20 pos.	none	1:10 pos. 1:20 neg.	
Blood Count	(See Chart I)	(See Chart I)	RBC: 4700000 WBC: 11700 Hb: 78%	none	none	none	none	RBC: 3300000 WBC: 11750 Hb: 69%	none	

Tolysin medication—30 gr./day

TABLE I—Continued

DIAGNOSIS	8891-TU ATROPHIC ARTHRITIS ACUTE TONSILLITIS		1150-LK ATROPHIC ARTHRITIS		917-LK ATROPHIC ARTHRITIS		1037-LK ATROPHIC ARTHRITIS		1127-LK ATROPHIC ARTHRITIS	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
UINALTIS	sl. tr. alb. no casts	sl. tr. alb. occas. hyal. cast	none	neg. alb. no casts or cells	no alb. no casts	ft. tr. alb. no casts	no alb. no casts	tr. alb. no casts	none	ft. tr. alb. no casts
P. S. P.	1 hr. 25% 2 hr. 30% 3 hr. 5%	1 hr. 29% 2 hr. 30% 3 hr. 15%	none	1 hr. 12% 2 hr. 12% 3 hr. 8%	1 hr. 33% 2 hr. 25% 3 hr. 13%	1 hr. 20% 2 hr. 20% 3 hr. 18%	1 hr. 35% 2 hr. 15% 3 hr. 10%	1 hr. 30% 2 hr. 20% 3 hr. 15%	none	1 hr. 5% 2 hr. 15% 3 hr. 15%
B. S. P.	5 min. 5% 30 min. 0% 60 min. 0% mal	5 min. 29% 30 min. 0% 60 min. 0% mal	5 min. 80% 30 min. 0% 60 min. 0% mal	5 min. 85% 30 min. 0% 60 min. 0% mal	none	none	none	none	none	5 min. 80% 30 min. neg. 60 min. neg. mal
Van Den Bergh	Direct: neg. Indirect: <0.2 mg.	Direct: neg. Indirect: <0.2 mg.	none	none	none	none	none	none	none	none
Icterus Index	4.0	4.0	none	5.5	3.0	1.0	1.5	5.0	none	5
Wassermann	negative	negative	doubtful	negative	none	negative	negative	none	negative	none
Kahn Reaction	negative	negative	doubtful	negative	none	negative	negative	none	negative	none
Blood Chemistry	Urea-N: 16 Creatin: 1.2 Uric Ac.: 4.81 Dextrose: 118.0	none	none	Urea-N: 8 Dextrose: 90	none	Cholesterol 180	Urea-N: 19 Dextrose: 90	Urea-N: 11 Dextrose: 90	none	Urea: 11 Dextrose: 90 Creatin: 1.8 Calcium: 9.0
Galactose Test	none	none	none	none	none	none	none	none	none	none
Urobilinogen	none	none	none	none	none	none	none	none	none	none
Blood Count	RBC: 3550000 WBC: 5050 Hb: 75%	RBC: 3400000 WBC: 6250 Hb: 78%	none	RBC: 4520000 WBC: 7700 Hb: 85%	none	none	none	none	none	RBC: 4040000 WBC: 5000 Hb: 80%

Tolysin medication — 30 gr./day

TABLE I—Continued

DIAGNOSIS	7664-TU ATROPHIC ARTHRITIS		7423-TU ATROPHIC ARTHRITIS		9836-TU ATROPHIC ARTHRITIS ENDOCRINE DYSGRASIA	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
URINALYSIS	ft. tr. alb. no casts	v. ft. tr. alb. no casts	t. tr. alb. no casts	ft. tr. alb. no casts	ft. tr. alb. no casts	no alb. no casts
P. S. P.	1 hr. 4% 2 hr. 3% 3 hr. 28%	1 hr. 5% 2 hr. 12% 3 hr. 27%	none	1 hr. 30% 2 hr. 30% 3 hr. 15%	none	1 hr. 30% 2 hr. 25% 3 hr. 18%
B. S. P.	5 min. none 30 min. neg. 60 min. neg./mal	5 min. q.n.s. 30 min. 0% 60 min. 0% mal	none	none	none	5 min. 30% 30 min. neg. 60 min. neg./mal
Van Den Bergh	Direct: neg. Indirect: <0.5 mg.	none	none	none	none	Direct: neg. Indirect: <0.2 mg
Icterus Index	6.0	4.0	4.0	4.0	none	6.0
Wassermann	negative	none	negative	none	negative	none
Kahn Reaction	negative	none	negative	none	negative	none
Blood Chemistry	Urea-N: 10 Uric Ac.: 3.4 Creatin: 1.4 Dextrose: 135	none	Urea-N: 14 Creatin: 1.13 Uric Ac.: 2.6 Dextrose: 121	none	none	Urea-N: 9 Uric Ac.: 3.6
Galactose Test	10 gm./5 hr.	13.8 gm./5 hr.	none	2.6 gm./5 hr.	none	1.96 gm./5 hr.
Urobilinogen	conc. positive 1:10 negative 1:20 negative	1:10 positive 1:20 negative	none	conc. positive 1:10 positive 1:20 negative	none	1:10 positive 1:20 negative
Blood Count	none	none	none	none	none	none

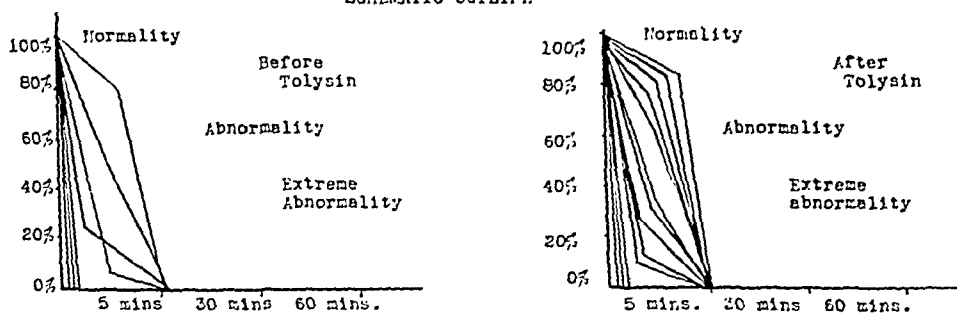
Tolysin medication—30 gr./day

lieved by medication, pending the institution of curative processes by nature and the physician, was pain. It is remarkable that, with the very small doses employed, only 3 per cent, and about the same in each series, were not relieved. The medication was obviously minimal in both series and apparently satisfactory. No toxicity was expected or observed.

It seemed to one of us (D. S.) that further, more detailed studies with extensive laboratory functional tests were indicated at higher dosage levels. Accordingly, a series of 23 cases, drawn from the wards of Temple University Hospital and Lankenau Hospital was studied quite carefully. It should be

GROUP FIVE - COMPARATIVE BROMULPHEALEIN FINDINGS

SCHEMATIC OUTLINE



Curves approximate only in terms of dye retention.

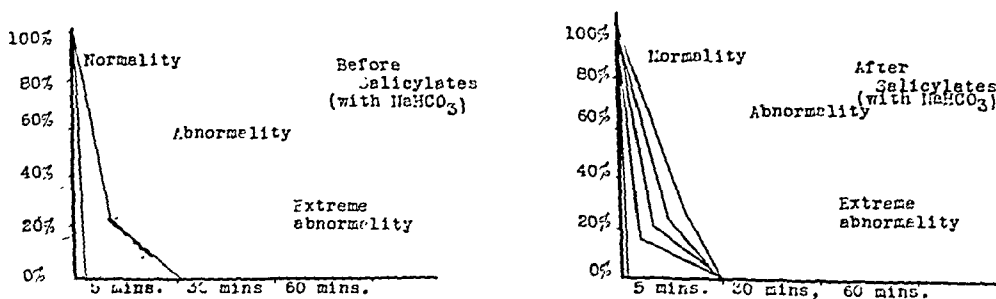


Chart II.

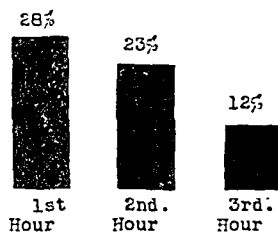
noted that this series was studied not for relief of pain, or for associated phenomena in arthritis, but principally to learn something of the organic functional response of arthritic patients to antirheumatic medication. To give the complete history and physical examination on each of these cases, in addition to the laboratory results, would consume entirely too much space; in fact, it is impossible to more than approximate the laboratory findings after medication was instituted. Almost 600 special laboratory, x-ray, blood pressure, and consultation examinations were made on the 23 cases. It must be pointed out that, in the tables subjoined, the tests representing work done during or after medication are always taken as the worst or least favorable test; and usually

represent three or more tests during that period upon that particular function. We have tabulated the laboratory findings in brief as follows:

Group I	13 cases under tolysin medication
Group II	5 cases under minimal salicylate and iodide medication
Group III	5 cases under standard salicylate and sodium bicarbonate medication
Group IV	Significant blood findings
Group V	Comparative B.S.P. findings
Group VI	Comparative P.S.P. findings

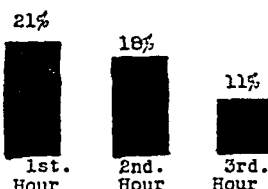
GROUP SIX -COMPARATIVE PHENOLSULPHONEPHTHALEIN FINDINGS

SCHEMATIC DIAGRAMS



No medication

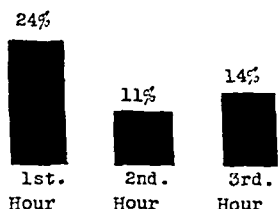
(Diagrams drawn to first two significant figures only of averages)



Tolysin medication

Averaged readings on 74 separate tests.

Moderate dosage of Salicylates with iodides, etc.



(Total of 23 cases for all renal function tests)

Full dosage of Salicylates used alone. (with NaHCO_3)

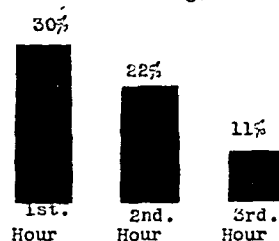


Chart III.

DISCUSSION OF TABULATED CASES

It will be noted in all the tables that it was impossible, due to the exigencies of hospital routine, to do every test upon every patient. Many more tests were done after, or during, medication than before. A consideration of all the findings gives at least temporary answer, however, to the question of the extent to which renal and hepatic functions are affected by standard moderate medication under the conditions of the study. On an average, three functional studies of the P.S.P., B.S.P., and icterus index were done for each one reported in the tables; but the graphic charts are inclusive of nearly all. An interesting point was that nearly all cases of arthritis due to infection show traces of albumin in the urine either before or after treatment, or both.

Case 9248.—T. U. (Table I), is interesting in that the case was clearly one of slightly impaired renal and hepatic function evidenced by the slightly raised icterus index and slightly subnormal P.S.P., and one positive indirect van den Bergh. Yet, under three weeks' medication with tolysin, the patient showed no significant change in the functional indices. Case 10726, T. U., showed a slightly raised icterus index, but no other signs. Case 7158, T. U., had all the evidence of focal infection over many years; but the E.K.G. did not reveal any wave abnormality; and the laboratory findings were normal. Case 8533, T. U., was of typical rheumatic origin. The heart was enlarged and there was an insufficiency of the mitral valve. E.K.G. showed extrasystoles and left axis deviation. The P.S.P. in this case was consistently low; but all other tests were approximately normal. On Case 8891, T. U., we did a *Mosenthal* test and found an ability to concentrate that was definitely diminished; yet the blood chemistry, P.S.P., and urinalysis showed insignificant changes. Liver function tests in the case were entirely normal. Case 1450, L. K., showed consistently low and declining P.S.P. without any other significant findings except a Wassermann and Kahn reaction first reported positive, then negative. In Case 1027, L. K., it was interesting to correlate the declining blood urea nitrogen with a rising P.S.P. output, the liver function test being essentially normal. Case 7664, T. U., showed definite progressive diminution in function of the kidney by P.S.P. test; but the icterus index improved while the galactose test was, and remained, poor, suggesting a renal factor. Case 7423, T. U., showed normal function test, a normal E.K.G., but a typical rheumatic history and was, in fact, a typical case of rheumatic disease. All of the above cases were treated, so far as analgesic medication was concerned, with 10 gr. of tolysin three times a day. So far as could be noted, there were no evidences of significant change in the laboratory tests after medication.

Group II cases were treated (Table II) upon a complex and somewhat irrational mixture known as elixir sodium salicylate, compound (N. F.) in amounts which would give a dose of about 18 gr. of the salicylate and $3\frac{1}{2}$ gr. of KI per day. At first it was thought to use a placebo in these cases; but the presence of a very small amount of the salicylate in the mixture gave it some effect and satisfied the patients. Case 863, L. K., was an interesting one because of the absolutely negative findings in the liver function test in the presence of clear abnormality elsewhere. Here the P.S.P. declined progressively; focal infection was obviously present in the tonsils; the blood urea nitrogen was definitely raised; yet the urine did not show much albumin and no sugar despite a very definite hyperglycemia. The case strikingly demonstrated the advisability of blood dextrose determinations in such cases. Case 1329, L. K., is interesting because, on physical examination, the liver was found considerably enlarged. The functional tests were, without exception, normal. Case 1122, L. K., is worth consideration since it was the only one in the series of 23 hospital cases in which a definitely abnormal B.S.P. was found after mild medication. Seven days later, however, the B.S.P. was normal as

TABLE II
GROUP TWO—TABLE OF CLINICAL TESTS ON FIVE HOSPITALIZED UNSELECTED CASES OF ARTHRITIS

DIAGNOSIS	863-LK		1323-LK		1122-LK		1170-LK		1112-LK	
	HYPERTROPIC ARTHRITIS BEFORE	HYPERTROPIC ARTHRITIS AFTER	HYPERTROPIC ARTHRITIS BEFORE	HYPERTROPIC ARTHRITIS AFTER	HYPERTROPIC ARTHRITIS BEFORE	HYPERTROPIC ARTHRITIS AFTER	HYPERTROPIC ARTHRITIS BEFORE	HYPERTROPIC ARTHRITIS AFTER	ATROPHIC ARTHRITIS BEFORE	ATROPHIC ARTHRITIS AFTER
URINALYSIS	v. ft. tr. alb. no casts	no alb. no casts	none	no alb. no casts	none	none	none	no alb. no casts	no alb. no casts	v. ft. tr. alb. no casts
P. S. P.	1 hr. 60% 2 hr. 18% 3 hr. 2%	1 hr. 45% 2 hr. 15% 3 hr. 14%	1 hr. 40% 2 hr. 30% 3 hr. 18%	1 hr. 30% 2 hr. 20% 3 hr. 20%	1 hr. 50% 2 hr. 20% 3 hr. 6%	1 hr. 40% 2 hr. 22% 3 hr. 12%	none	1 hr. 18% 2 hr. 20% 3 hr. 8%	1 hr. 32% 2 hr. 27% 3 hr. 20%	1 hr. 30% 2 hr. 20% 3 hr. 10%
B. S. P.	5 min. 35% 30 min. 0% 60 min. 0%	5 min. 45% 30 min. neg. 60 min. neg./mal	none	5 min. 100% 30 min. neg. 60 min. neg./mal	5 min. q.n.s. 30 min. 0% 60 min. 0%	5 min. 50% 30 min. 20% 60 min. 0% altally neg./mal	none	5 min. 95% 30 min. neg. 60 min. neg./mal	none	5 min. 90% 30 min. neg. 60 min. neg./mal
Van Den Bergh	none	none	none	none	none	none	none	none	none	none
Icterus Index	none	5.5	none	4.5	none	5.5	none	4.5	7.0	4.0
Wassermann	anticomplementary	negative	none	negative	none	negative	negative	none	four plus	plus 1
Kahn Reaction	negative	negative	none	negative	none	negative	negative	none	one plus	plus 2
Blood Chemistry	Urea-N: 30 Dextrose: 180.0	Urea-N: 24 Dextrose: 165	none	Urea-N: 18 Dextrose: 95	Dextrose: 70	Cholesterol: 250	none	none	Urea-N: 6 Dextrose: 95 Creatin: 1.4 Chlorides: 140 Cholesterol: 320	Dextrose: 95
Galactose Test	none	none	none	none	none	none	none	none	none	none
Urobilinogen	non	none	none	none	none	none	none	none	none	none
Blood Count	RBC: 4040000 WBC: 6600 Hb: 75%	RBC: 4320000 WBC: 6800 Hb: 80%	none	RBC: 1080000 WBC: 6800 Hb: 80%	none	none	none	RBC: 4180000 WBC: 6000 Hb: 85%	none	none

Sodium Salicylate about 18 gr. plus KI about 31.5 gr.

TABLE III
GROUP THREE—TABLE OF CLINICAL TESTS ON FIVE HOSPITALIZED UNSELECTED CASES OF ARTHRITIS

DIAGNOSIS	8000-TU		8320-TU		9313-TU		9838-TU		3693-TU	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
URICACIDEMIA	v. fl. tr. alb. no casts	v. fl. tr. alb. no casts	v. sl. tr. alb. no casts	v. sl. tr. alb. no casts	v. sl. tr. alb. no casts	v. sl. tr. alb. no casts	v. fl. tr. alb. no casts	v. fl. tr. alb. no casts	none	none
P. S. P.	1 hr. 54°C 2 hr. 39°C 3 hr. 27°C	1 hr. 47°C 2 hr. 27°C 3 hr. 12°C	1 hr. 87.8°C 2 hr. 78°C 3 hr. 13°C	1 hr. 39°C 2 hr. 10°C 3 hr. 10°C	1 hr. 27°C 2 hr. 8°C 3 hr. 17°C	1 hr. 12°C 2 hr. 17°C 3 hr. 15°C	1 hr. 12°C 2 hr. 10°C 3 hr. 12°C	1 hr. 12°C 2 hr. 10°C 3 hr. 12°C	none	none
R. S. P.	none	none	none	none	5 min. 25°C 30 min. neg. nor- mal	5 min. 25°C 30 min. neg. nor- mal	5 min. 25°C 30 min. neg. nor- mal	5 min. 25°C 30 min. neg. nor- mal	norma	norma
Van Den Bergh	none	none	negative <0.2 mg.	negative <0.2 mg.	negative <0.2 mg.	negative <0.2 mg.	negative <0.2 mg.	negative <0.2 mg.	negative <0.2 mg.	negative <0.2 mg.
Icterus Index	1.0	0.0	none	3.0	3.0	0.0	0.0	1.0	3.0	3.0
Wassermann	negative	negative	negative	negative	none	negative	negative	none	none	none
Kahn Reaction	negative	negative	negative	negative	none	negative	negative	none	none	none
Blood Chemistry	none (Basal metab. minus 16)	none (Basal metab. minus 8)	Urea-N: 14.8 Uric. Ac.: 2.6 Creatin: 1.3 Dextrose: 101 Cholesterol 200	Urea-N: 12 Creatin: 1.6 Uric. Ac.: 1.3 Dextrose: 132 Cholesterol 290	none	Urea-N: 13 Creatin: 1.3 Uric. Ac.: 2.7 Dextrose: 80	Urea-N: 13 Creatin: 1.2 Uric. Ac.: 2.5 Dextrose: 82	Urea-N: 18 Creatin: 1.8 Uric. Ac.: 3.0 Dextrose: 139	Urea-N: 19 Creatin: 1.8 Uric. Ac.: 3.0 Dextrose: 139	
Galactose Test	none	none	none	none	none	none	1.30% ems./5 hr.	none	none	none
Urobilinogen	none	none	none	none	none	none	none	none	none	none
Blood Count (last)	RBC: 4780000 WBC: 10500 Hb: 87%	RBC: 4200000 WBC: 9750 Hb: 72%	none	RBC: 2900000 WBC: 12950 Hb: 63%	RBC: 3320000 WBC: 8750 Hb: 63%	none	none	none	none	none

Average dose Sodium Salicylate 80 gr./day. Sodium Bicarbonate 80 gr./day.

Average dose Sodium Salicylate 80 gr./day. Sodium Bicarbonate 80 gr./day

TABLE IV
GROUP FIVE. BROMSULPHALEIN TEST

(Before Tolysin):		RETENTION AT END OF		
CASE	DATE	5 MIN.	30 MIN.	60 MIN.
TU 7158	5/11/32	q.n.s.	neg.	neg.
TU 8533	8/29/32	25%	neg.	neg.
TU 8891	7/15/32	5%	neg.	neg.
LK 1450	1/14/33	80%	neg.	neg.
TU 7664	5/ 3/32	q.n.s.	neg.	neg.
(After Tolysin):				
TU 9248	8/16/32	50%	neg.	neg.
TU 6676	5/ 7/32	q.n.s.	neg.	neg.
TU 10726	{ 11/ 2/32	35%	neg.	neg.
	{ 11/ 9/32	8%	neg.	neg.
TU 7158	5/24/32	q.n.s.	neg.	neg.
TU 8891	8/ 5/32	28%	neg.	neg.
LK 1450	{ 1/20/33	85%	neg.	neg.
	{ 1/27/33	5%	neg.	neg.
	{ 2/ 3/33	8%	neg.	neg.
LK 1127	{ 1/ 4/33	85%	neg.	neg.
	{ 1/11/33	75%	neg.	neg.
	{ 1/18/33	60%	neg.	neg.
	{ 1/25/33	80%	neg.	neg.
TU 7664	5/18/33	q.n.s.	neg.	neg.
TU 9836	11/12/32	30%	neg.	neg.
(Before Sodium Salicylate and Sodium Bicarbonate):				
none	none	none	none	none
(After Sodium Salicylate and Sodium Bicarbonate):				
TU 8000	{ 7/25/32	15%	neg.	q.n.s.
	{ 8/ 1/32	20%	neg.	q.n.s.
TU 9343	{ 7/15/32	25%	neg.	q.n.s.
	{ 8/ 5/32	18%	neg.	q.n.s.
TU 9836	8/30/32	25%	neg.	neg.
TU 5693	{ 5/ 5/32	q.n.s.	neg.	neg.
	{ 5/18/32	q.n.s.	neg.	neg.
(Before Sodium Salicylate Mixture):				
LK 863	12/16/32	35%	neg.	neg.
LK 1122	12/16/32	q.n.s.	neg.	neg.
(After Sodium Salicylate Mixture):				
LK 863	{ 12/23/32	neg.	neg.	neg.
	{ 12/30/32	45%	neg.	neg.
	{ 1/ 6/33	30%	neg.	neg.
LK 1329	{ 1/11/33	85%	neg.	neg.
	{ 1/18/33	100%	neg.	neg.
	{ 1/25/33	95%	neg.	neg.
LK 1179	{ 12/28/32	80%	80%	neg.
	{ 1/ 4/33	90%	neg.	neg.
	{ 1/11/33	90%	neg.	neg.
	{ 1/18/33	60%	neg.	neg.
LK 1112	{ 1/25/33	95%	neg.	neg.
	{ 12/23/32	85%	neg.	neg.
	{ 12/28/32	50%	neg.	neg.
	{ 1/ 8/33	90%	neg.	neg.
LK 1122	{ 1/11/33	90%	neg.	neg.
	{ 12/23/32	50%	20%	neg.
	{ 12/30/32	80%	neg.	neg.

were all other tests at all times; and as a consequence the significance of the findings, if any, cannot be determined. Case 1179, L. K., had several interesting features, including an abnormal B.S.P. became normal after a few days' stay in the hospital; a consistently low P.S.P. unassociated with albumin or casts in the urine; and a normal icterus index with improving B. S. P. It was interesting to note that during this period his liver, by physical signs, progressively enlarged. We offer no explanation. This single group was, in a loose sense, a control group. Nothing abnormal was expected or noted.

Group III (5 cases) represented standard dosage with sodium salicylate and sodium bicarbonate, antiarthritic medication widely recognized in hospitals. All of these cases showed one or more interesting points. All had a definite rheumatic fever history and in all the diagnosis of acute rheumatic fever was justified. None showed any significant change in laboratory findings under the above medication, and particularly was the liver function free from such change. In three cases there was a definite trend downward (rather than upward as claimed by the protagonists of sodium bicarbonate) in the P.S.P. test (Cases 8529, T. U.; 9343, T. U.; 9836, T. U.), but this change was commonly later reversed. The E.K.G. findings showed cardiac involvement in two cases (Cases 8000, T. U., 5693, T. U.).

The subject of blood studies has not been mentioned up to this point, but in these 23 cases some blood work was done on each, and a great deal of work was done on many. In some, the so-called "Schilling hemogram" was constructed to indicate comparatively the complete count (repeated from five to ten times) together with an estimate of the shift in the nuclear structure of the white cells "to the left" or "to the right." In this regard we have been very much interested in the recent publication by Steinbrocker and Hartung¹⁸ on the "filament-nonfilament count" of arthritis as an aid in differential diagnosis. We have scarcely space to go into an analysis of these cases as viewed from such an angle, but hope to be able to do so in a subsequent publication. Solely to illustrate the constant tendency to hypochromic anemia in these cases, we have appended a few rough diagrammatic charts of the course of the red cells, hemoglobin and white count. It is our preliminary opinion that the hypochromic anemia, which almost invariably occurs in these cases, is best treated when infection is quiescent and hemolysis has ceased.²³ We should like to call attention to the article by Robertson, Swalm, and Konzelmann¹⁹ in which an excellent summary of liver function tests is given. We have made no attempt to use the recently suggested Lichtman²⁰ test of hepatic function because of the difficulty of obtaining betahydroxyphenyleinchoninic acid for the preparation of standards.

SUMMARY

No essential difference was noted in the clinical response of 120 unselected cases of arthritis to minimal doses of tolysin or sodium salicylate in grain-for-grain equivalent dosage. The presence of potassium iodide, cimicifuga, gel-

seminum, and such medicaments did not seem to add to, or detract from, the effects noted.

The thirteen cases under 30 gr. of tolysin daily, carefully studied upon hospital wards, showed no signs of liver involvement which could be confirmed by any of the common laboratory functional tests frequently repeated, and the kidney signs were in conformity with the usual findings of a slight albuminuria in arthritis regardless of medication. In this matter, our experience coincides with that of Chace, Myers and Killian²¹ who remarked over ten years ago, after careful study: "In the cases which we employed, neither cinchophen nor neocinchophen produced proteinuria or intensified it when already present. . . ."

The series of five cases upon very small doses of salicylates (Group II) failed to differ materially from any of the others from a laboratory standpoint.

The series of five cases upon standard salicylate medication averaging more than 80 gr. of sodium salicylate per day (Group III) together with sodium bicarbonate, differed not at all from the preceding cases in the character of their functional tests.

The entire study failed to show any effects of so-called toxicity from anti-rheumatic medication in moderate or small dosage during the period of study and with the dosages employed. We wish at this point to express our great appreciation to Doctors Robertson, Konzelmann, Kline and Bortz for their co-operation; and to Miss Schubert and Miss Hohl for assistance in the pathologic work.

CONCLUSIONS

1. Moderate dosage of tolysin and sodium salicylate with bicarbonate, produced no appreciable changes in the laboratory tests for total renal and hepatic function in arthritic cases under the conditions of our study.

2. Hypochromic anemia is an almost constant concomitant of acute and chronic nontraumatic arthritis.

3. The widely accepted treatment of arthritis, involving accurate diagnosis; anti-etiological measures; anatomic, physiologic and psychic rest; the regulation of bodily and endocrine chemistry, is best charted and checked by careful functional laboratory studies.

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A STUDY OF THE OTTO TEST FOR STRYCHNINE*

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ONE of the earliest tests devised for the detection of the presence of strychnine was proposed by Otto¹ in 1846. This test consists of treating a small quantity of strychnine with a few drops of concentrated sulphuric acid. When a small crystal of potassium dichromate is moved about in this liquid a deep violet color changing to a dirty green is produced.

A study in this laboratory² has shown that the Mandelin test for strychnine is given by a number of substances other than strychnine. A study was therefore undertaken in order to determine whether or not the Otto test was more specific for strychnine than was the Mandelin test.

A record has been made of a number of substances which produce a color similar to that given by strychnine with $\text{H}_2\text{SO}_4 - \text{K}_2\text{Cr}_2\text{O}_7$. Wormley³ states that curarine and cod liver oil give a similar test. Witthaus⁴ lists geissospermin as giving the same test as strychnine, while aniline at first gives the test but soon changes to a black. A number of substances are listed by Wormley³ which will interfere with the test. These include morphine, quinine, sugar, brucine, and tartar emetic.

In working with crude drugs, Fuller⁵ has found that the petroleum ether residues from gelsemium and yohimbe give the strychnine test.

Mameli⁶ made a study of the interference of certain substances employed in therapeutics on the Otto color reaction. He found a number of drugs which more or less interfered with the test. The amounts of the drugs used in the tests, however, were not given.

PROCEDURE

Preparation of Solutions to Be Tested.—The organic compounds used in this investigation were prepared in the proper solvent so that 1 c.c. was equivalent to 1 mg. of the compound. The strychnine solution was of the same strength.

Procedure of the Tests.—Three tests were made on each compound. For the first, 1 c.c. of the solution of the organic compound was evaporated and the usual test was made and any color noted. The second test was made in the same manner except that 1 c.c. of strychnine was evaporated with the same volume of the organic compound and the test made on the mixture. The third test was carried out as above, except that 5 c.c. of the impurity were mixed with the strychnine.

The results of the tests when the organic compounds were tested alone are given in the pages which immediately follow: The name is given first and directly opposite is given the color reaction.

*From the University of Colorado.

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ALKALOIDS AND ALKALOIDAL SALTS

<i>Organic Substances</i>	<i>Color Reaction</i>
Apomorphine	Brownish green
Berberine	Brown to black
Brucine	Orange
Colchicine	Orange, acid alone
Cotarnine hydrochloride	Orange
Cryptopine	Violet, acid alone
Delphinine	Reddish brown, acid alone
Gelseminine	Reddish brown
Hydrastine	Yellow, trace of violet
Papaverine	Light violet, acid alone
Piperine	Violet brown
Sanguinarine nitrate	Greenish black, acid alone
Solamine	Yellow, acid alone
Strychnine	Violet to blue to orange
Veratrine	Orange to red, acid alone

No Color Reaction.—Aconitine, atropine, belladonnine, caffeine, cinchonidine, cinchonine, cocaine, codeine, daturine, dionine, emetine, ergotine, beta-eucaine hydrochloride, heroine, homatropine, hyoseyamine, morphine, narceine, nicotine, pelletierine, phenacaine, physostigmine, pilocarpine, pseudopelletierine, quinidine, quinine, scopolamine, sparteine, theobromine, theophylline.

AMINO ACIDS AND DERIVATIVES

Glycyltryptophane	Dirty green
Phenylglycine	Violet brown
Tryptophane	Dirty green, trace purple

No Color Reaction.—Acetylphenylglycine, alpha-alanine, para-aminophenylglycine, arginine, asparagine, aspartic acid, *dl*-benzoylalanine, betaine hydrochloride, creatine, creatinine, diiodotyrosine, edestine, ethylglycollate, glutamic acid, glycine, hippuric acid, isoleucine, leucine, para-nitrophenylglycine, alpha-phenylalanine, beta-phenylalanine, tyrosine, *dl*-valine.

ALIPHATIC ACIDS

No Color Reaction.—Aconitic acid, adipic acid, *dl*-alpha-aminoacetylacetic acid, alpha-bromopropionic acid, beta-bromopropionic acid, formic acid, fumaric acid, levulinic acid, maleic acid, malic acid, malonic acid, mesaconic acid, mucic acid, palmitic acid, propionic acid, stearic acid, succinic acid, tartaric acid, tartronic acid, trichloroacetic acid.

ALIPHATIC ACID SALTS, ESTERS, AND DERIVATIVES

No Color Reaction.—Ethyl oxalate, ethyl succinate, isoamyl propionate, isobutyl acetate, isobutyl isothiocyanate, methyl isothiocyanate, sodium formate, sodium oxalate, thallous formate, thallous malonate, triacetin, tributyrin.

ALIPHATIC ALCOHOLS AND KETONES

No Color Reaction.—Acetylacetone, cetyl alcohol, dulcitol, erythritol, ethylene glycol, isobutyl alcohol, isopropyl alcohol, mannitol, melissyl alcohol, methyl heptenone, octyl alcohol, phorone, trichlorobutyl alcohol.

SUGARS

No Color Reaction.—Arabinose, galactose, glucose, lactose, levulose, maltose, *d*-mannose, melezitose, raffinose, rhamnose, sucrose, xylose.

UREA AND URIC ACID DERIVATIVES

Allylphenylthiocarbamide	Brown, streaks of violet
Amytal (isoamylethylbarbituric acid)	Light green
Triphenylguanidine	Brown to green

No Color Reaction.—Allylthiocarbamide, allylthiourea, acetyl methylurea, allantoin, alloxantine, barbital (diethylmalonylurea), barbituric acid, biuret, *dl-n*-butylthiourea, dibromobarbituric acid, diphenylthiourea, guanine hydrochloride, ipral (calcium ethylisopropylbarbiturate), luminol (phenylethylbarbituric acid), peralga (aminopyrinediethylbarbiturate), thiobarbituric acid, thiourea, urea, urethane, uric acid.

GLUCOSIDES

Aesculin	Brown, trace violet
Arbutin	Reddish violet, acid alone
Colocynthin	Yellow, acid alone
Digitalin	Reddish brown, acid alone
Salicin	Magenta, acid alone
Saponin	Brown, acid alone

No Color Reaction.—Amygdalin, convallamarin, elaterin, phloridzin, picrotoxin, santonin.

MISCELLANEOUS ALIPHATIC COMPOUNDS

Oenanthal	Green
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No Color Reaction.—Acetal, acetaldoxime, acetamide, acetoxime, aldehyde ammonia, aminoguanidine bicarbonate, bromoform, *ter*-butyl bromide, chitin, chloral hydrate, chloral urethane, chloropierine, dimethylglyoxime, hexachloroethane, hexamethylenetetramine, iodoform, isobutylbromide, methylglyoxal sodium bisulphite, monochlorohydrine, nitrosodiethylamine, oxamide, pinacol hydrate, propionamide, sulphonal, thialdine, tribromohydrine, trichloroacetylchloride, trimethylene bromide, trional, veronal.

BENZENE AND TOLUENE DERIVATIVES

Azoxybenzene	Brown
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No Color Reaction.—Ortho-bromochlorobenzene, para-bromochlorobenzene, ortho-bromonitrobenzene, meta-chloronitrobenzene, ortho-chloronitrobenzene, para-chloronitrobenzene, para-chlorotoluene, ortho-dichlorobenzene, 2-5-dichloronitrobenzene, iodosobenzene, isopropylbenzene, meta-nitrotoluene, ortho-nitrotoluene, para-nitrotoluene, mesitylene, styrene, para-xylenesulphonic acid.

ANILINE AND DERIVATIVES

ortho-Anisidine	Violet black, acid alone
Benzanilide	Deep violet
ortho-Benztoluide	Reddish violet
para-Bromoaniline	Reddish violet to brown
ortho-Phenetidine	Deep violet, acid alone
para-Phenetidine	Light violet, acid alone
Phenyl-beta-diphenylamine	Green, acid alone
ortho-Tolidine	Deep orange

No Color Reaction.—Acetoanilide, acetyl-para-anisidine, acetyl-ortho-methyltoluidine, acetyl-para-methyl-toluidine, acetylphenetidine, acetyl-ortho-toluidine, acetyl-para-toluidine, meta-acetylidine, aminoazobenzene, 2-aminotoluene-4-sulphonic acid, 2-aminotoluene-5-sulphonic acid, 4-aminotoluene-2-sulphonic acid, aniline, meta-anisidine, para-anisidine, para-benztoluide, para-bromoacetanilide, meta-bromoaniline, ortho-bromo-aniline, chloramine, meta-chloroaniline, ortho-chloroaniline, para-chloroaniline, 1, 2, 4-dichloroaniline, 2, 5-dichloroaniline, 1, 2, 4-dinitroaniline, exalgine, meta-nitroaniline, para-nitroaniline, meta-nitrodimethylaniline, para-nitrodimethylaniline, nitroso-dimethylaniline, 1, 2, 3-nitrotoluidine, 1, 2, 4-nitrotoluidine, 1, 3, 4-nitrotoluidine, meta-toluidine, ortho-toluidine, para-toluidine, tribromoaniline, trinitroaniline, 1, 2, 4-xylydine, 1, 3, 4-xylydine.

PHENOLS AND DERIVATIVES

meta-Aminophenol	Dirty green
ortho-Aminophenol	Light violet, acid alone
para-Aminophenol	Blue black, acid alone
5-Benzalmino-2-cresol	Yellowish green, acid alone
para-Benzalaminophenol	Green, acid alone
Catechol	Green, acid alone
ortho-Chloromercuriphenol	Yellowish green
para-Nitrophenol	Pink
Phloroglucinol	Slate to green

No Color Reaction.—Acetyl-meta-aminophenol, acetyl-para-aminophenol, benzoylthymol, para-bromophenol, carvacrol, ortho-chlorophenol, para-chlorophenol, meta-cresol, ortho-cresol, para-cresol, 3, 5-dibromo-ortho-cresol, 2, 4-dichlorophenol, dimethylhydroresorcinol, 2, 3-dinitrophenol, 2, 4-dinitrophenol, 2, 6-dinitrophenol, meta-nitrophenol, ortho-nitrophenol, orcinol, phenol, picric acid, pyrogallie acid, tetrabromo-ortho-phenol, thymol, tribromophenol, trichlorophenol, xylenol.

AROMATIC ACIDS

Anisic acid	Brown, tinge of violet
Benzilic acid	Magenta, acid alone
Cinchophen	Light green
Cumaric acid	Red to green
Diiodosalicylic acid	Dark yellow
Diphenylacetic acid	Yellow to dark green
Mandelic acid	Brown

No Color Reaction.—Acetylsalicylic acid, meta-aminobenzoic acid, para-aminobenzoic acid, *dl*-aminophenylacetic acid, anthranilic acid, arsanilic acid, meta-bromobenzoic acid, ortho-bromobenzoic acid, para-bromobenzoic acid, meta-chlorobenzoic acid, ortho-chlorobenzoic acid, para-chlorobenzoic acid, cinnamic acid, gallic acid, 5-iodosalicylic acid, para-mercuri-chlorobenzoic acid, metanilic acid, naphthionic acid, meta-nitrobenzoic acid, ortho-nitrobenzoic acid, para-nitrobenzoic acid, quinic acid, salicylic acid, tannic acid, terephthalic acid, ortho-toluic acid, para-toluic acid.

AROMATIC ACID DERIVATIVES

Benzyl benzoate	Brown
Neocinchophen (ethyl-6-methyl-2-phenylquinoline-4-carboxylate)	Brownish green

No Color Reaction.—Benzamide, benzoic anhydride, butyl benzoate, cumarin, ethyl benzoate, ethyl salicylate, isoamyl benzoate, isoamyl salicylate, isobutyl benzoate, methyl benzoate, methyl cinnamic ester, methyl salicylate, nicotinic acid nitrate, meta-nitrobenzoyl chloride, para-nitrobenzoyl chloride, phenyl salicylate, phthalimide.

AROMATIC ALDEHYDES, ETHERS, ALCOHOLS, AND KETONES

Benzalacetophenone	Brown
ortho-Nitrobenzaldehyde	Red, acid alone
5-Nitrosalicylaldehyde	Dirty green
Salicylaldehyde methyl ether	Greenish brown, acid alone
Saligenin	Pink, acid alone
Vanillin	Brown

No Color Reaction.—Para-aminoacetophenone, anisaldehyde, anisole, benzalacetone, benzhydrol, benzophenone, ortho-bromoanisole, para-bromoanisole, ortho-bromonitrobenzaldehyde, 1, 2, 5-bromosalicylaldehyde, ortho-chlorobenzaldehyde, isophthalaldehyde, meta-methoxysalicylaldehyde, methylacetophenone, para-nitroanisole, phenetole, phthalic acid aldehyde, piperonal, salicylaldehyde, tetramethyldiaminobenzophenone, para-tolylaldehyde.

HETEROCYCLIC COMPOUNDS

Isatin	Brown
Piperidine	Brown

No Color Reaction.—Acridine, antipyrine, dimethyl-pyrone, furoic acid, 6-nitroquinoline, oxyquinoline, quinaldine, quinoline, skatole, succinimide.

HYDROAROMATIC COMPOUNDS

Carvene	Yellow-brown, acid alone
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No Color Reaction.—*d*-Borneol, *l*-borneol, *dl*-camphor (natural), camphor (synthetic), camphoric^o acid, camphor sulphonic acid, carvenone, limonene, menthol, quercite, terpineol, terpenyl acetate.

NAPHTHALENE AND ANTHRACENE DERIVATIVES

Alizarin	Reddish violet, acid alone
Dibromoanthracene	Light brown
beta-Naphthol	Green with black streaks
Naphtholmethyl-alpha-ether	Green, acid alone
alpha-Naphthylamine	Bluish grey, acid alone
alpha-Naphthylaminoazobenzene	Deep violet, acid alone
Nitroso-beta-naphthol	Brown, acid alone

No Color Reaction.—Acenaphthene, acet-alpha-naphthalide, acet-beta-naphthalide, alpha-bromonaphthalene, 1, 5-dinitronaphthalene, beta-naphthalenesulphonic acid, naphthalic anhydride, beta-naphthylamine, alpha-naphthylisocyanate.

MISCELLANEOUS AROMATIC COMPOUNDS

Amarine	Green
Benzocatechin	Dark green
Benzoïn	Green, acid alone
Benzylphenylhydrazine	Rose violet
Fluorene	Dark green
Phenanthrene	Greenish brown
Phenanthrenequinone dioxime	Reddish brown, acid alone
Phenolphthaleïn	Reddish orange, acid alone
meta-Phenylenediamine hydrochloride	Black, acid alone
Tetrabromophenolphthaleïn	Violet red, acid alone
Thymolphthaleïn	Magenta, acid alone
Triphenylmethane	Brown, traces of violet

No Color Reaction.—Abeitic acid, adrenaline, benzil, 2, 4-dinitrophenylhydrazine, diphenyl, isoamylphenylhydrazine, isoeugenol, ortho-nitroacet-meta-xylidide, meta-nitrobenzhydrazide, para-nitrophenylhydrazine, phenylhydrazine hydrochloride, quercite, rheumatine (saloquinine salicylate), salvarsan, thiosemicarbazide, ortho-tolunitrile, para-tolunitrile, para-tolylisothiocyanate, para-tolylquinolinesulphate, para-tolylthioquinanthrene, tumeric.

The color reactions for the tests where an equal amount of impurity was added to the strychnine were recorded, and also the reactions where five times the amount of impurity was added; but the listing of these would require too much space. Therefore, only those compounds which caused the violet color to be completely masked will be given. With the remainder of the compounds, many gave no interference, whereas others gave more or less interference.

Organic compounds which completely covered up the Otto test for strychnine when present in equal amounts were: Aesculine, meta-aminophenol, azoxybenzene, benzoic acid, benzohydrol, para-benzotoluide, dionine, diphenylacetic acid, diphenylthiourea, beta-naphthol, alpha-naphthylamine, alpha-naphthylisocyanate, phenanthrenequinone dioxime, rheumatine, thymolphthaleïn, orthotolidine.

Organic compounds which completely covered up the Otto test when present in amounts five times that of strychnine, in addition to those listed above were: Acet-alpha-naphthalide, acet-beta-naphthalide, meta-acetxylidine, acetyl-meta-aminophenol, acetyl-para-aminophenol, acetyl-para-anisidine, amarine, aminoazobenzene, para-aminophenol, apomorphine, benzalacetophenone, 5-benzal-amino-2-cresol, para-benzalaminophenol, benzophenone, berberine, brucine, orthochloromercuriphenol, colocynthin, cotarnine hydrochloride, diiodosalicylic acid, elaterin, heroine, 5-iodosalicylic acid, isoamylsalicylate, isoeugenol, morphine, beta-naphthalenesulphonic acid, naphthionic acid, naphtholmethylalpha-ether, beta-naphthylamine, neocinchophen, meta-nitrophenol, para-nitrophenol, nitroso-beta-naphthol, oenanthol, phenacaine, phenanthrene, phloridzin, phloroglucinol, piperine, salicin, sanguinarine nitrate, thiosemicarbazide, paratolylisothiocyanate, triphenylguanidine, tryrosine, veratrine.

When a study is made of the preceding data, it will be noted that there were twenty-three compounds which gave some shade of violet, lavender, or purple. Some of these were very similar to the strychnine test and might be mistaken for

it. Of these twenty-three tests, there were eleven which gave the violet, lavender, or purple color with the sulphuric acid alone. The color with the acid would account for some of the positive tests which are obtained with such reagents as Fröhde's, Mandelin's, and the dimethylaminobenzaldehyde reagent.

In general, the compounds giving a test similar to that of strychnine belong to no definite group. The alkaloids and aniline derivatives, however, gave the largest number of positive tests.

There were a few organic compounds which interfered with the test. Many of these, however, would be removed by different organic solvents in a carefully conducted analysis.

There were somewhat fewer positive tests for strychnine given by the Otto test than by the Mandelin test, but the number of substances which interfered with the former test was somewhat greater.

CONCLUSIONS

1. A large number of organic compounds have been tested with the Otto-Strychnine reaction. A number of compounds have been found which give the same test as does strychnine.

2. The interferences caused by organic compounds as impurities have been determined.

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STUDIES OF THE MECHANISM OF PAIN IN PEPTIC ULCER*

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SEVERAL theories have been advanced by various authors in an attempt to explain the mechanism of pain in peptic ulcer. These theories may be discussed under the following headings:

1. Increased muscle tension
2. Chemical irritation of the ulcer
3. The compression theory of Kinsella

1. *Muscle Tension Theories.*—Hurst in 1911¹ considered that pain in peptic ulcer was due to strong peristaltic waves advancing against a pylorus that fails to relax, "achalasia." Glaessner, Kreuzfuchs, and others thought that spasm of the pylorus and of the duodenal cap was the most important factor in ulcer pain.

Carlson, Guinzburg and associates, Hardt, and others made kymographic tracings from intragastric balloons. These tracings showed that pain was felt at the time of or just after maximum hunger contractions.

Reynolds and Mc Clure² observing ulcer patients fluoroscopically found various types of motor phenomena during the painful phase in patients with peptic ulcer. The motor activity varied from quiescence to intense activity.

Ortmeyer,³ on the other hand, observed that no change occurred in the character of gastric contractions during relief of pain by alkalies.

Wilson⁴ examined fluoroscopically 16 duodenal ulcer patients while in pain. He found contracted, spastic duodenal caps in all of these patients. By manual manipulation he managed to partially fill the duodenal cap in 13 of the 16 patients. Relaxation shown by filling of the cap was immediately followed by pain relief in all of the 13 cases. Wilson concluded with the hypothesis "that relief of pain is due to relaxation of musculature of the caput and conversely, that pain is due to overaction of the duodenal caput with or without abnormal local contraction or implication of the pyloric sphincter."

Apparently increased tissue tension, due to contraction of muscle or increased peristalsis, plays a rôle in the mechanism of pain in patients with peptic ulcer.

2. *The Theory of Chemical Irritation of the Ulcer.*—That pain in duodenal ulcer cases can be caused by exposing the ulcer to the action of hydrochloric acid has been shown by Bonninger.⁵

Palmer⁶ showed that when a sufficient quantity of 0.5 per cent hydrochloric acid was injected into the stomach of a patient with peptic ulcer, in the active stage of the disease, typical ulcer pain was produced in 95 per cent of his cases.

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After the onset of pain, aspiration of the entire contents of the stomach resulted in immediate relief. Ingestion of alkalis sufficient to considerably reduce the acidity of stomach contents also resulted in pain relief. He further showed that as the ulcer patient became symptom-free, the pain-sensitizing action of 0.5 per cent HCl. becomes less. Palmer regards this reaction as an index of healing in ulcer.

Hurst, and Reynolds and Mc Clure failed to cause ulcer pain by injection of hydrochloric acid.

Despite this, it seems apparent that in peptic ulcer under certain conditions, the acidity of the gastric contents is a factor in the mechanism of ulcer pain.

3. *Compression of Sensory Nerves.*—Kinsella⁷ in a very complete monograph criticizes previous attempts to explain the pain mechanism in ulcer as being too limited in their scope. He introduced a concept whereby ulcer pain is explained primarily by compression of sensitized sensory nerves in and around the ulcer base.

Kinsella points out that a moderate amount of compression is exerted by the edema and inflammatory exudate on the intrinsic sensory nerves around the ulcer base. Peristalsis and muscle spasm following a meal, augment this tension. Furthermore, at a specific interval after eating, the vascular channels of the antral and pyloric regions become engorged, thereby adding still another factor to increase local tension. Compression, therefore, is attained by the summation of the three factors; inflammatory edema, increased muscle tone or spasm, and localized vascular engorgement, all occurring in and around the ulcer at a specified time after meals. Kinsella concludes that ulcer pain is caused by compression of sensitized nerves around the ulcer at that time. Ulcer pain relief depends on decrease in tissue tension. Alkalis, when ingested, relax the musculature of the pylorus and duodenum. This was shown roentgenographically by Carman and by Kinsella. Food ingestion also decreases ulcer pain. As the stomach fills, the entire gastric vascular bed becomes engorged. In order to supply part of the required blood to the entire stomach, the vascular engorgement of the ulcer area necessarily diminishes. As local engorgement decreases, so also does the tissue tension in the ulcer region, and hence the compression pain is relieved.

I. INDIVIDUAL SENSITIVITY TO PAIN

Another factor is probably of prime importance in the problem. This consists in the individual susceptibility of the patient to pain.

Libman⁸ pointed out in 1926 that individuals may vary widely in their sensitivity to a standard pain-producing stimulus. He stressed the problem in relation to coronary artery disease and also ulcer.

Later Crohn⁹ reported that in hyposensitive individuals, peptic ulcer caused little or no pain. Hypersensitive individuals, on the contrary, definitely experience the pain caused by their ulcer. Individual sensitivity to pain seems so important a factor in interpreting symptomatology that it was decided to reinvestigate the relationship of factors associated with ulcer pain in the light of individual pain sensitivity.

The subjective symptom of pain in disease seems dependent upon the sensitiveness of the individual. Thus the phlegmatic Nordic may respond less actively to a standard painful stimulus than the hypersensitive Latin. A simple clinical test was introduced by Libman so that patients can easily be examined as to their individual pain sensitiveness. The test is performed as follows: The examiner, using the side of the ball of his thumb, makes firm pressure first on the mastoid, then on the styloid process between the tip of the mastoid and the angle of the jaw of the patient. Both sides are examined. The mastoid is the control point. The response of the patient to styloid pressure constitutes an index of pain sensitivity. A normal response is interpreted by a facial contortion indicating pain; hypersensitive patients wince, jerk the head away, and often cry out with pain; hyposensitive patients have slight or no change in their facial expression despite a strong increase in thumb pressure. In these studies patients were classified as normal; hypersensitive, 1-plus and 2-plus; hyposensitive, 1 minus and 2 minus. A significant fact in working with Libman's valuable clinical sign is that psychic manifestations or so-called "nervousness" and pain sensitivity are not directly related. That is, an individual may be insensitive to pain as determined by styloid pressure and yet be markedly psychoneurotic.

The relationship of typical peptic ulcer symptomatology to pain sensitivity of the individual as determined by Libman's sign was investigated. All patients were classified in two groups according to subjective symptoms. Patients were considered typical in symptomatology when they complained of localized epigastric pain definitely related to meals, pyrosis, night pain, possibly vomiting; these symptoms often being relieved by the ingestion of alkalis and food. The symptomatology was considered atypical if pain was vague or absent or if substitution symptoms, such as gas or heart burn or pressure alone were present. Cases were also considered atypical where the diagnosis of ulcer was made after investigation of the patient for painless hematemesis, painless vomiting, and for the vague painless dyspeptic symptoms just described. Three cases of sudden perforation with no previous gastric symptoms were included.

Material.—Ninety-nine patients with duodenal ulcer were studied and 120 clinical observations made. Sex distribution: Male 80, and female 19. Youngest twenty-one, and oldest sixty-seven years. Duration of ulcer symptoms: Shortest three weeks, and longest twenty years.

The presence of ulcer was proved roentgenographically in all cases and confirmed by operation in 12.

Observations.—In this group of 99 patients taken as a whole, sensitiveness as tested by Libman's sign was distributed as follows: Normal 28, hypersensitive 31, and hyposensitive 40. The preponderance of individuals hyposensitive to pain is possibly to be expected in this dispensary group, more exposed and accustomed as they are to rigorous conditions of life.

In eliciting the history we found that 74 (75 per cent) of our 99 patients had symptoms at some time which were typical of peptic ulcer. This group of ulcer cases with typical symptoms when examined for pain sensitivity by Lib-

TABLE I

PEPTIC ULCER SYMPTOMATOLOGY AND PAIN SENSITIVENESS. PATIENTS EXAMINED, 99

SYMPTOMS	CASES	PAIN SENSITIVENESS
Typical	74 (75%)	26 (35%) Hypersensitive
		23 (31%) Normal
		25 (34%) Hyposensitive
Atypical	25 (24%)	5 (20%) Hypersensitive
		5 (20%) Normal
		15 (60%) Hyposensitive

man's test were equally distributed in the three groups: Normal (23 per cent), hyposensitive (25 per cent), and hypersensitive (26 per cent) (Table I). Twenty-five ulcer cases with atypical symptoms had little or no pain. In these atypical cases we rather expected to find hyposensitiveness to pain according to Libman's test. Actually 15 cases (60 per cent) were hyposensitive, 5 cases (20 per cent) were hypersensitive, and 5 cases (20 per cent) were normal when tested.

II. PAIN DUE TO THE COMPRESSION FACTOR AS TESTED BY PERCUSSION TENDERNESS

During the examination of patients in the active stage of peptic ulcer, it was noted that percussion in the epigastric region revealed a localized point of tenderness. This area, in size no larger than a half dollar, was definite in its demarcation from the remaining relatively insensitive epigastrium. A tender point in the epigastrium similar to the above has been mentioned by Mac Lean, Crohn, and Bolton in writing on the physical diagnosis of ulcer. Bolton called it the "Hyperalgesic Point." To elicit percussion tenderness, fairly heavy percussion is made on the relaxed epigastrium of the supine patient. If a point of definite and sharply localized tenderness is not found, percussion is repeated after the patient has been asked to inspire and hold the breath in deep inspiration. In calling this sign "positive" it is essential to observe that the tenderness must be definite and sharply localized in area. Patients having slight, absent, or mild diffuse tenderness, are considered "negative."

The mechanism involved is not entirely clear. It is probable that in percussing, the plexor impulse is transmitted by vibration through the abdominal wall and intermediate tissues directly to the inflamed region surrounding the ulcer base. This vibratory impulse affects the inflamed nerves already under tension in the indurated tissues around the ulcer. It is also possible that the intragastric pressure and the tissue tension may be increased directly or reflexly by this maneuver. This sudden mechanical disturbance results in the production of momentary pain directly under the pleximeter finger. The use of percussion in the erect position as a diagnostic measure in ulcer has been described by Erhlich in 1923 and Kohn in 1928.

Ninety-nine patients were examined for tenderness to percussion using the technic just described. A positive reaction was found in most patients examined during the stage of ulcer activity. Patients examined in the remission or inactive, symptom-free stage of the disease were usually negative to "percussion tenderness."

The statistics are as follows:

Fifty-five (46 per cent) observations were made while the patients were in the active symptomatic stage of the disease. Forty-seven (85 per cent) of these responded positively when examined by percussion tenderness. Only 8 (15 per cent) active cases were negative to percussion. Six of these were hyposensitive when examined by Libman's test. In these individuals, possessing apparently a high threshold of pain sensitiveness, it is likely that percussion is an inadequate stimulus for eliciting pain.

Arbitrarily we considered that one week or longer of freedom from ulcer symptoms placed the patient in the remission or inactive stage of the disease. Sixty-five (54 per cent) patients were examined while symptoms were not active. Some of these had previously been examined in the active stage. Of these 65 inactive cases, 46 (71 per cent) were negative for "percussion tenderness." Despite inactivity at the time of examination, 19 (29 per cent) showed positive "percussion tenderness." Many of these had been recently active (one to four weeks previously). It is possible that the pain producing factor, which can be induced by percussion, is still active despite the absence of ulcer symptoms.

Table II shows the relationship of "percussion tenderness" to symptomatology, the Palmer Test, and pain sensitivity. Patients showing positive percussion tenderness usually had typical symptoms (88 per cent). Seventy-

TABLE II

THE RELATIONSHIP OF "PERCUSSION TENDERNES" TO SYMPTOMATOLOGY, ACTIVITY, PAIN SENSITIVENESS AND PALMER'S ACID TEST

MATERIAL: 120 EXAMINATIONS ON 99 CASES OF PEPTIC ULCER

"PERCUSSION TENDERNES"	SYMPTOMATOLOGY	ACTIVITY	SENSITIVENESS	ACID TEST
	Typical 57 (88%)	Active 48 (74%)	Hypersens. 23 (36%)	Pos. 20 (45%)
Pos. 65 (55%)	Atypical 8 (12%)	Inact. 17 (26%)	Normal 21 (32%)	Neg. 24 (55%)
	Typical 33 (60%)	Active 7 (13%)	Hypersens. 12 (24%)	Pos. 5 (12%)
Neg. 55 (46%)	Atypical 22 (40%)	Inact. 48 (87%)	Normal 14 (26%)	Neg. 37 (88%)
			Hyposens. 29 (52%)	

four per cent were in the active stage of the disease at the time of examination. The acid test meal was positive in 45 per cent. Sensitivity to pain in this group was equally divided into the normal, hyper-, and hyposensitive groups.

On the other hand, of the patients with negative "percussion tenderness," 87 per cent were in the inactive remission stage of the disease at the time of examination, 88 per cent were negative to the acid test and hyposensitive patients predominated over normal and hypersensitive in the ratio of 2 to 1. From this study it appears that a positive response to percussion tenderness is a valuable aid in the physical diagnosis of peptic ulcer especially in a patient with typical ulcer symptoms who is in the active stage of the disease.

III. PAIN DUE TO CHEMICAL IRRITATION PRODUCED BY HYDROCHLORIC ACID

As stated above Palmer found that hydrochloric acid produced pain in cases of peptic ulcer. Palmer's technic was carried out in 80 patients. Briefly this consisted in evacuating the contents of the fasting stomach and then instilling 200 c.c. of 0.5 per cent hydrochloric acid through the Rhexuss tube. Similar amounts of acid are instilled at half-hourly intervals for a total of 600 c.c. Pain, similar to usual ulcer pain of the individual, when produced within one and one-half hours after beginning the test indicates a positive response.

Observations.—Thirty-four patients were examined while in the active stage of the disease. Of these 21 (62 per cent) had a positive reaction, that is, typical ulcer pain was induced by the acid. Palmer reported that over 90 per cent of active cases gave a positive response to this test. Forty-six cases in

TABLE III
ACTIVITY OF ULCER SYMPTOMS AND PALMER'S ACID TEST MEAL

	ACTIVE STAGE OF ULCER	INACTIVE REMISSION STAGE
Patients Examined	34	46
Positive	21 (62%)	3 (7%)
Negative	13 (38%)	43 (93%)

TABLE IV
SENSITIVENESS TO PAIN AND PALMER'S ACID TEST MEAL IN ACTIVE ULCER CASES

	PALMER TEST		PALMER TEST
Positive	21	Negative	13
	<i>Sensitiveness to Libman's Sign</i>		
Normal	6	Normal	6
Hypersensitive	4	Hypersensitive	2
Hyposensitive	11	Hyposensitive	5

the remission stage were examined by the Palmer acid test. Only 3 (7 per cent) gave a positive response (Table III). The pain sensitivity of patients with positive Palmer tests was noted. No definite relationship was found in this small series (Table IV).

SUMMARY AND CONCLUSIONS

1. The rôle of muscle tension, chemical irritation, and nerve compression in the mechanism of ulcer pain is discussed.

2. The importance in the problem of individual sensitiveness to pain is stressed.

3. Studies in patients with ulcer in the active and remission stages show that (a) percussion tenderness (muscle tension and nerve compression) is present in 85 per cent of patients during the active period of the disease. This is a helpful sign in the physical diagnosis of ulcer. (b) Patients with typical ulcer history may be hypersensitive, hyposensitive, or normally sensitive to pain. (c) Patients with atypical ulcer symptomatology, however, are mostly hyposensitive to pain. (d) The Palmer Acid Test Meal (chemical irritation)

is positive in the majority of ulcer patients during the active stage and negative in the remission stage.

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15 EAST TENTH STREET.

FOOD ALLERGY AS A COMMON PROBLEM*

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THERE are two general types of food allergies, the fortunate and the unfortunate. The former comprise those who were lucky enough to become sensitive to foods which are eaten only intermittently or occasionally. They have no difficulty in attributing their untoward symptoms to the correct cause. Instinctively they thereafter avoid the offending foods and are relieved of their allergic manifestations. As we shall see this forms a large group and these "fortunate allergies" are usually considered nonallergic.¹

The second or unfortunate group suffer the misfortune of becoming sensitive to staple foods, foods eaten frequently, usually daily. Chronic exposure, resulting in more gradual evolution of symptoms, a realization that in the past all of these foods have been eaten with impunity, and an understanding that they are not naturally poisonous, all combine to keep the sufferer ignorant of the cause of his symptoms. The man who experiences an explosive outbreak of urticaria with the advent of each strawberry season finds no difficulty in discovering its etiology. Thereafter he avoids strawberries. His friend, with subacute or chronic symptoms and sensitive to wheat is less likely to spontaneously discover the source of his trouble.

The allergic group representing from 7 to 10 per cent of the entire population is recruited chiefly from the unfortunates.² The fortunates are usually considered as in the nonallergic group because they have at some time in the

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past solved their own problem and at the time of questioning they have no allergic symptoms. I use the terms fortunate and unfortunate, since so far as we know at present it is to a great extent just an element of chance to what one will become sensitive.

Other terms might be equally appropriate, as minor allergy and major allergy; or acute and subacute or chronic; or simple and complex. Each of these classifications is open to some criticism. It is sufficient that we understand that in the minor or fortunate group we are dealing with transient or passing episodes while in the major or unfortunate group we are faced with the problem of an established malady.

MINOR ALLERGY AND TRANSIENT ALLERGY

I have felt that we should be able to reach a clearer understanding of food allergy were we to direct more attention to an understanding of the simple or transient episodic manifestations of the phenomenon. With this in mind I have made a study of a number of so-called nonallergies, searching for evidences of allergic reactions in the past. Analysis of only the first 100 questionnaires has been completed and while the series is not sufficiently large to justify final conclusions, the observations are most interesting. Among the 100 so-called nonallergies, we found histories of former acute allergic manifestations in 61 per cent. In an additional 11 per cent the story was suggestive but not definite. The remainder were negative. If to the 61 per cent we add the 7 to 10 per cent of frank allergies, we may infer from this series that upward of 70 per cent or more are allergic at one time or another. Of the 61 per cent, 46 gave positive histories for food allergy and almost without exception they could name the foods causing symptoms.

A comparison of the foods recognized by the patient as causing minor allergy with those found by testing to be responsible for major allergy is of interest (Table I). With the major allergies, I have found by skin tests³ that the following foods are chief offenders, in order of frequency; wheat, milk, bean, egg, and Irish potato. The minor allergies, on the other hand, name the following foods in order of frequency as chief offenders; cucumber, watermelon, strawberry, tomato, onion, and cabbage. In this group wheat does not appear at all, egg and milk only once, potato and bean only twice each. Most of the remaining foods are such as are eaten occasionally.

I find evidence in my studies that the normal physiologic tendency is toward a gradual loss of specific sensitizations. There has in the past been a rather general impression that once one becomes sensitized this specific sensitization tends to persist throughout life. But students of allergy have in the last few years had the opportunity of watching sensitizations appear and disappear, to be replaced by new ones. Likewise, serial retesting will show that foods and other allergens, once positive, sometimes become negative, while others formerly negative become positive. It is but a simple step farther to postulate that the natural tendency of specific sensitization is to a gradual disappearance. The rapidity of this disappearance depends of course upon the degree or intensity of the sensitization and also upon whether the reaction

is kept active by repeated or continued contact with the sensitizing allergen. If avoidance of contact could be carried out sufficiently long with every allergen, the probability is that our sensitizations would eventually disappear. New ones of course may take their places. Allergists for example have almost universally concluded that food allergy is better handled through the method of avoidance than with attempts at desensitization either orally or parenterally. Prolonged contact intensifies the allergic reaction and delays recovery. I refer here more particularly to food allergy. Perennial pollen desensitization is an apparent exception which, however, I believe that we can reconcile to the hypothesis.

As examples of transient allergy the following may be cited:

Mr. V. in the summer of 1929 experienced attacks of colitis with cramping pains and diarrhea each time that he ate peaches. Almonds which belong to the same biologic group as the peach caused the same reaction. One year previously he had experienced typical vasomotor rhinitis each time following the drinking of rye whisky. None of these three ingestants produced symptoms prior to or subsequent to these years.

Mr. H. experienced hives after eating tomatoes during the World War and for two years thereafter. Since then he has been able to eat them without reaction, usually eating them about twice daily.

Miss R., aged fifteen, had had hives at age ten for a period of two months attributed to strawberries. She avoided them for a time but can eat them now without difficulty.

Mr. R. experienced urticaria from strawberries in childhood. He eats them now without discomfort.

Mrs. B. found that during one summer watermelon consistently produced vomiting and diarrhea, but since then, she has had no trouble.

Mrs. F. eight years ago found that watermelon produced angioneurotic edema of the hands and tongue. Even the juice of watermelon on the hands produced swelling. Watermelon now causes no symptoms.

Miss W. observed that several years ago strawberries caused abdominal cramps and nausea. She avoided them for several years and this year has taken them without discomfort.

Mrs. M. three years ago had hives in the summer time, cause unexplained. Since then she has had no return.

Mrs. C. experienced eczema in childhood involving the hands only and lasting for three months. This was presumably due to chocolate. She has had no return of trouble until within the last few months when the eating of chocolate produces urticaria and cardiac palpitation, often with headaches. Plums consistently produced diarrhea a year ago: none before or since.

Mrs. B. experienced diarrhea from cut corn in childhood. She eats corn without difficulty now.

Mr. G. several years ago regularly had sour stomach and heartburn from the eating of cucumbers. He now eats them without difficulty.

The transient allergic rarely needs advice or study by the physician. He himself realizes the etiology of his symptoms and cures himself by avoidance. Not all lose their sensitization after a period of avoidance. Not a few report the reappearance of symptoms each time they eat the offending food. This group obtains relief by one of three methods, (a) outgrowing the specific sensitization, (b) continued avoidance, and (c) unconscious incidental changes in their routine by which they unwittingly are removed from the causative

agent. This happens more frequently in inhalant and contact allergy than in food allergy. A lady in her forties had had asthma in childhood which, as she stated, she outgrew. She was sensitive to cat hair and remarked that while they had had cats in the house in childhood she had always despised them and that she had had none for years. She had relieved herself intuitively by avoidance.

MAJOR OR FRANK ALLERGY

The physician is, however, more frequently faced with the problem of determining the offending food in the major or frank allergic.

Here several procedures may be followed. Realizing that the frank allergic is more likely to be sensitive to the foods usually eaten, the staples, the doctor may place his patient on an elimination diet or trial diet from which the common offenders have been deleted.⁴ This would work well were it not that in addition to the staples the chronic allergic is also usually sensitive to one or more of the infrequent offenders, as is the minor allergic. Since these usually show up positive on skin testing, indeed more frequently than do the staple offenders, we find it more logical and more frequently successful to perform preliminary skin testing on the basis of which infrequent and frequent offenders may be promptly eliminated without guesswork. It is quite true that one may be sensitive to an allergen which fails to produce positive skin reaction just as one may show positive skin reaction to an allergen which fails to cause trouble. But in the great majority there is a correlation between positive skin reaction and symptoms. It has been conservatively estimated that skin testing will give only 50 per cent of reliable information on allergenic foods.⁵ But if this procedure diminishes the amount of necessary subsequent work by 50 per cent it is well worth employing and is indeed the foundation procedure for subsequent investigative superstructure.

TESTING METHODS

Sensitization tests may be performed either by the scratch or cutaneous method or by intracutaneous or endermal testing or by passive transfer. The last is only occasionally required, particularly in the study of infants or individuals with such generalized dermatitis that there is not sufficient space for skin testing or in those with a general urticarial tendency in which all tests show up positive. In this event blood is obtained from the patient, the serum is diluted and filtered through porcelain and introduced intracutaneously into the skin of the nonallergic recipient. Later, the recipient is tested in the passively sensitized sites by the intracutaneous method, with control tests applied to areas of his nonsensitized skin.

The customary procedure in the majority of cases, however, is the scratch test and the intracutaneous test. Both have their advantages. The former possesses the advantage of safety with the disadvantage of diminished accuracy. The latter is more sensitive but occasionally dangerous because of its extreme sensitiveness. Both methods should be combined. If one would avoid the rare anaphylactic reaction, one will never do intracutaneous tests without preliminary scratch test. This is our routine procedure. As far as foods are

concerned, scratch tests are first applied with the individual foods. The positive scratch foods are removed from the intracutaneous test boards and the remainder of the series is then repeated with endermal tests. In order to reduce the requisite number of intracutaneous tests, group intracutaneous reactions are obtained, using solutions containing two or more allergens grouped together in accordance with their genetic or biologic relationships.⁶

Where intracutaneous tests are done without preliminary scratch testing, the potentialities for untoward reactions are so great that one must use very dilute intracutaneous solutions. In this case 1/5,000 concentration is usually selected. The intracutaneous test is about 100 times more sensitive than the scratch.⁷ In our own work I use a scratch concentration of 1/50. If in our intracutaneous work we were to use 1/5,000 concentration or 100 times dilution over our scratch reaction, we would find that the intracutaneous test is no more sensitive than the scratch. This is the advantage of preliminary scratch testing. If the scratch reaction has been negative, one can proceed intracutaneously with a solution at least ten times more sensitive. This implies a 1/500 instead of 1/5,000 concentration. As a matter of fact we have no hesitancy in our own work in using 1/250, provided the preliminary scratch test was negative.

In short, if one is using 1/5,000 concentration for intracutaneous testing, this is no more accurate than the scratch method. But if one will use a more concentrated intracutaneous solution after preliminary scratch testing, results will be more accurate due to the finding of an additional number of low grade positive reactions. Intracutaneous extracts in which foods are grouped in accordance with biologic relationships diminish the requisite number of needlings. It is not safe to employ groups in scratch testing since each additional food in the group increases the likelihood of false negative reactions.

As a rule the intracutaneous test will show as positive some of the reactions that were negative by the cutaneous test. However, not infrequently foods and other allergens will show up positive by the scratch method, being at the same time negative intracutaneously. This is another reason why the combination of the two methods promotes greater accuracy.

Assuming that we are dealing with a case of food allergy and that the sensitization studies have shown positive reactions, we will proceed to place the patient on a special diet from which the positive reactors have been eliminated and to which adequate substitutes have been added to provide the requisite food values.

TRIAL DIETS AND THE FOOD DIARY

If, in a food allergic the program fails of results, the next logical step is the trial or the elimination diet.

The diets that have been proposed in the literature are based on the principle that in major allergy foods more frequently eaten are the more frequent offenders, and that these are especially likely to give false negative reactions on skin testing. If one will pay attention to the borderline reaction and the delayed positive, the latter assumption will not be found to be true as often

as is generally believed. But the first assumption is quite valid. Series of alternative elimination diets have therefore been proposed which provide for the elimination of the common ingestants but which do not take into consideration the undoubted fact that the major allergic is usually sensitive to one or more relatively infrequent foods as well as to one or more common foods. And several of the infrequent foods appear on the elimination diets. For this reason I object to the use of the elimination diets without preliminary skin testing. Instead, I prefer my trial diet,³ which, however, must be individualized for each patient. It has a double basis, the elimination of staple foods and, in addition, the avoidance of those foods to which the patient was found on trial to be sensitive. It is built up on a foundation list of foods to which in my experience both groups of patients are infrequently sensitive and to which are added other foods to which the patient has been found by testing to be nonsensitive. Naturally, if the patient has been found sensitive to a member of the foundation list that also is deleted.

There are a few food allergies in whom the offending food cannot even be found after a combination of the sensitization studies and the trial diet. In this case the food diary becomes requisite. It is only of value where the symptoms are intermittent and I have found from experience that it is much more easily followed if the patient is already on a restricted diet such as the trial diet. A list is kept, daily, of all foods eaten as well as of any occurrences out of the usual routine of the day. Days on which symptoms are manifested are checked and a comparison of these checked days with the foods eaten on that day and the preceding day and with the events of these two days occasionally enables one to determine the etiologic factor.

Thus we see that the treatment of the food allergic goes along in a logical sequence based first upon the sensitization reactions, modified as need be by the trial diet, and finally checked, if necessary, with the food diary.

DISCUSSION WITH THE PATIENT

I have omitted discussion of the first and one of the most important procedures of the examination, discussion with the patient. Note that I do not state "taking the history." Discussion with the patient in allergy involves much more than routine history taking. It is because it should follow as well as precede the allergic examination that I discuss it now. Much information can often be obtained from discussion with the patient of his past allergic history prior to the institution of testing, but a surprising amount of additional information is often acquired from a continuation of the discussion after the patient has been made acquainted with the list of positive reactions. This will refresh his memory on many hitherto unremembered incidents of his past experience and will show a surprisingly increased correlation between the positive skin tests and the patient's own experiences. Discussion should include a careful inventory of the family allergic history including not only asthma, hay fever and urticaria but also eczema, sick headaches and food upsets. The same series of maladies should be investigated in the patient. Also symptoms suggestive of allergic indigestion or colitis. One should bear in mind that

the manifestations of allergy are often obscure and bizarre, and one should be on the watch for unusual symptoms which can be fitted into the picture, both at the time of the preliminary questioning and at the final discussion as well as at subsequent follow-up discussions that may be had from time to time. The following cases represent some of the unusual manifestations of food allergy.

A young lady of twenty-four complained of lack of energy. If she stays up as late as 11 o'clock she feels dead the next day. She says she could sleep indefinitely. However, at night she does not sleep soundly. She had had nocturia since childhood. She had had her appendix removed six years previously in an effort to relieve her symptoms and for two months prior to examination she had been fed increasing doses of thyroid. Her metabolic rate had been minus 16 and the asthenia has been ascribed to thyroid deficiency. However, the thyroid intake had been increased to 12 gr. daily with no improvement but with symptoms of thyrotoxicosis, daily diarrhea, trembling, palpitation, nervousness and vertigo.

Discussion with the patient brought out that each year she had a spell of urticaria which she believes is due to strawberries and tomato, that her sister develops a vasomotor rhinitis when near cats, and that her brother sneezes many times each morning when he arises. She gave only borderline reactions to the legumes, chocolate, wheat, banana, and ginger. All medication including thyroid, was discontinued. The foods mentioned were eliminated. Five days later she reported that she had more pep than she had had for months, and now, five months later, she still reports herself full of energy, free from nocturia, sleeping well and relieved of a neuritis that had been bothering her for several months.

Note that she complained of no allergic symptoms. They were brought out only after discussion. The following cases also bring out this point.

A man of thirty-five, complained of a low grade dermatitis of three years' duration, worse in the autumn, of heartburn and constipation, and of easy fatigability. As he said, he awakened tired in the morning and had to drag himself out of bed. He was seeking relief for his indigestion. Discussion with him developed that he had had seasonal hay fever in childhood from age eight to twenty-one and occasional headaches of undetermined origin in childhood. In former years before the development of the chronic heartburn, he had had intermittent attacks of heartburn brought on definitely by cucumber, tomato, and strawberry. He was found sensitive to 14 foods including milk, peas and beans among the staples and apple, pear, peanuts, celery, cantaloupe, cucumber, berries, chocolate, tomato and banana among the occasionals. On dietary restrictions his skin rash, lack of energy and indigestion disappeared, and although he had been obstinately constipated for years, his bowels became regular. Two years later he reported that except for one interval when he had strayed from his diet he remained entirely well.

Miss M., complained of extreme dysmenorrhea and some low grade indefinite indigestion. It was found that she sneezed a great deal. Her hay fever was not sufficiently severe to justify treatment. She knew the cause to be dust. No cause other than a possible allergy was found for her indigestion.

TABLE 1*
SURVEY OF ALLERGENIC FOODS

	POSITIVE REACTORS IN MAJOR ALLERGY PER CENT	FOODS BLAMED IN MINOR ALLERGY	FOOD DISLIKES
<i>Vegetable Foods:</i>			
Wheat	24.0	0	
Rye	3.5	0	
Barley	3.5	0	
Oat	4.5	0	
Rice	2.5	0	
Corn	4.0	2	
Cocoonut	5.5	0	
Pineapple	1.5	2	1
Onion	6.5	9	5
Asparagus	5.5	1	1
Banana	4.5	2	2
Ginger	2.0	0	
Fig			1
Buckwheat	2.0	0	
Rhubarb			4
Walnut	3.0	1	
Pecan	0	0	
Spinach	5.5	2	17
Beet	0	0	
Swiss chard	2.0	0	
Turnip	4.0	3	13
Cabbage	5.0	8	11
Sauerkraut			1
Cauliflower	3.0	0	4
Mustard	5.5	0	1
Radish	2.0	0	
Raspberry	3.0	0	1
Strawberry	0.1	10	2
Apple	3.5	2	2
Pear	3.0	0	
Almond	4.5	1	
Prune	7.0	1	
Cherry	7.5	1	
Apricot	5.5	0	
Peach	5.0	2	1
Pea	3.0	1	1
Kidney bean	12.0	2	2
Peanut	2.5	2	
Blackeye pea	4.0	0	3
Lima bean	6.0	0	
Bean (string)	3.5	0	
Lentil	4.0	0	
Lemon	2.5	0	
Grapefruit	3.0	1	2
Orange	5.0	0	
Grape	3.5	0	
Cotton seed	6.0	0	
Okra	2.0	0	9
Cocoa	4.0	4	
Tea	0.6	0	
Celery	8.0	0	
Carrot	3.0	0	10
Parsnip	4.0	0	
Parsley	0	0	
Huckleberry		1	
Olive			4
Sweet potato	1.5	1	1
Tomato	7.0	10	3
Pepper	0.6	3	3
Potato	9.5	0	

*The order in which the vegetable foods appear follows the botanical or genetic classification.

TABLE I*—CONT'D

	POSITIVE REACTORS IN MAJOR ALLERGY PER CENT	FOODS BLAMED IN MINOR ALLERGY	FOOD DISLIKES
<i>Vegetable Foods:</i>			
Eggplant	7.0	0	3
Coffee	7.0	0	
Pumpkin	0	1	
Squash	3.5	1	8
Cantaloupe	5.0	7	
Pickles			1
Cucumber	2.5	13	5
Watermelon	0	13	
Lettuce	3.5	0	
<i>Animal Foods:</i>			
Beef	2.0	0	
Veal	2.0	1	
Milk	14.0	1	
Buttermilk			1
Butter			1
Cheese		1	1
Egg	9.5	1	1
Chicken	3.4	0	1
Pork	1.0	2	
Lamb	3.0	0	8
<i>Sea Foods:</i>			
Clam		1	2
Shrimp		1	
Oyster			
Crab			
Lobster		1	
Fish		2	4
Herring		1	1
Sardine		1	
Caviar			1
Anchovies			2
<i>Miscellaneous:</i>			
Whisky		1	
Sweets		3	1
Acids		2	
Meats		3	
Condiments		1	
Mayonnaise		1	
Fruits		1	2
Brains		0	5
Tongue		0	2

*The order in which the vegetable foods appear follows the botanical or genetic classification.

Her chief complaint was dysmenorrhea which she described as intense cramps with sometimes, fainting spells on the first and third days.

She was found sensitive to wheat, cotton seed, corn, and some incidental foods and was advised to avoid these during the week prior to, and through the duration of her period. She did this with relief of pain.

Miss H., also subject to ragweed hay fever, suffered from headache over the left eye, only during the catamenia. She was also found sensitive to certain foods and discovered that by avoiding them during the week prior to and through the catamenia she was relieved of these headaches.

FOOD DISLIKES

It has been suggested that a history of food dislikes suggests a sensitization to the undesired food, that dislike for a food to which the patient is unknowingly sensitive constitutes a protective reaction.⁵ My own experience has been rather the reverse, that most allergies will say that they are very fond of the particular food which is responsible for their symptoms. Only occasionally have I found evidence of a correlation between dislike and sensitization.

In my series of 100 so-called nonallergics, 46 showed evidence of food allergy and 66 expressed food dislikes. Most complained of the taste, some of the appearance, and some explained that they continued to taste them for many hours after eating. The list of foods disliked is altogether different from that of foods causing minor allergic manifestations, and includes, in order of frequency, spinach, turnip, cabbage, carrot, okra, lamb and squash, with a scattering of others (Table I).

Food dislikes as well as food disagreements were usually multiple. In no instance was there entire agreement between foods disliked and foods responsible for symptoms. In four instances one among several foods and in five instances two among several foods corresponded in both groups. We may conclude, therefore, that while dislikes may occasionally be indicative they cannot be relied upon.

There is an old superstition or better tradition that certain combinations of foods which are individually harmless become harmful to certain individuals in an allergic way. This tradition crops out in my series. Whether it is an actual synergism or merely a coincidence remains for future investigation to determine. Combinations mentioned are such as milk and fruit, orange juice and ice cream, cake and cabbage, fish and milk, deviled crab and ice cream. Each of these foods eaten separately caused no reaction nor did the individual constituents of the following special dishes to which symptoms were attributed: crab flake salad, frozen fruit salad, potato salad. In these salads the cotton seed oil presumably present in the salad dressing may have been the responsible factor.

CONCLUSION

I trust that in discussing these many ramifications of the problem of food allergy I have not made it appear discouragingly complex. It is a complex problem which can only be made simple by an adequate understanding of it. The points which I have discussed should enable one to avoid most of the many pitfalls. Too many doctors and patients alike feel that the skin test is the last court and conclude if the results of skin testing fail to relieve, that allergy is not a factor.

I would emphasize particularly the need for frequent follow-ups, particularly in those who are not doing as well as one thinks they might. It is surprising how often new factors are found to crop up. The variability in sensitization from time to time and the factor of interaction with nonspecific causes are chiefly responsible for this. One should bear in mind at all times that the

allergic state is never static. It is intimately bound up with the problem of nutrition and indeed with the biochemical activities of the process of life itself. Representing as it does changing responses to a changing environment it is not at all surprising that supervision must often be continued over a prolonged period before all factors are finally brought under adequate control.

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808 PROFESSIONAL BUILDING

THE INACTIVATION OF GROWTH HORMONE*

AS A RESULT OF INADEQUATE REFRIGERATION

H. S. RUBINSTEIN, M.D., BALTIMORE, MD.

IN CARRYING out experiments dealing with the brain weight-body weight ratio¹ it became necessary to use anterior pituitary extracts as growth stimulators. In the course of this work it was found that while fresh extracts possessed this quality, the effect was lost when refrigeration was inadequate. It is the purpose of this communication to report on the inactivation of growth hormone as experienced in this laboratory. An extract of the anterior lobe of the pituitary gland was prepared by a modification of the Putnam, Teel and Benedict² technic so that 2 c.c. of the final product represented one gram of the fresh anterior lobe. Such extract when used *fresh* showed a definite growth-stimulating effect.¹ When ready for use as fresh extract this preparation was distinctly alkaline to phenolphthalein and possessed a P_H of 8.3.

Twenty-one adult female white rats (*Mus norvegicus albinus*) were used in these experiments and these were arranged so that only litter mates were used as controls. Litters were used which contained four or five females of which two received growth hormone, one received no injections, and the remaining one or two, injections of meat extract.

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Upon intraperitoneal administration of this extract in the dosage of 2 c.c. daily (except Sunday) growth effects became quite noticeable. The controls injected with meat extract and those uninjected failed to increase in size.

It was found, however, that after a growth increase for two or three weeks the rats injected with the same preparation began to lose weight. A fresh extract, alkaline in reaction, was substituted and the next week the animals again gained weight. This weight increase and subsequent weight loss was noted repeatedly showing that the growth-stimulating effect of the extract used was lost after two or three weeks (Chart I).

A study of the inactivated extract revealed that its alkalinity had decreased to such an extent that it was no longer alkaline to phenolphthalein.

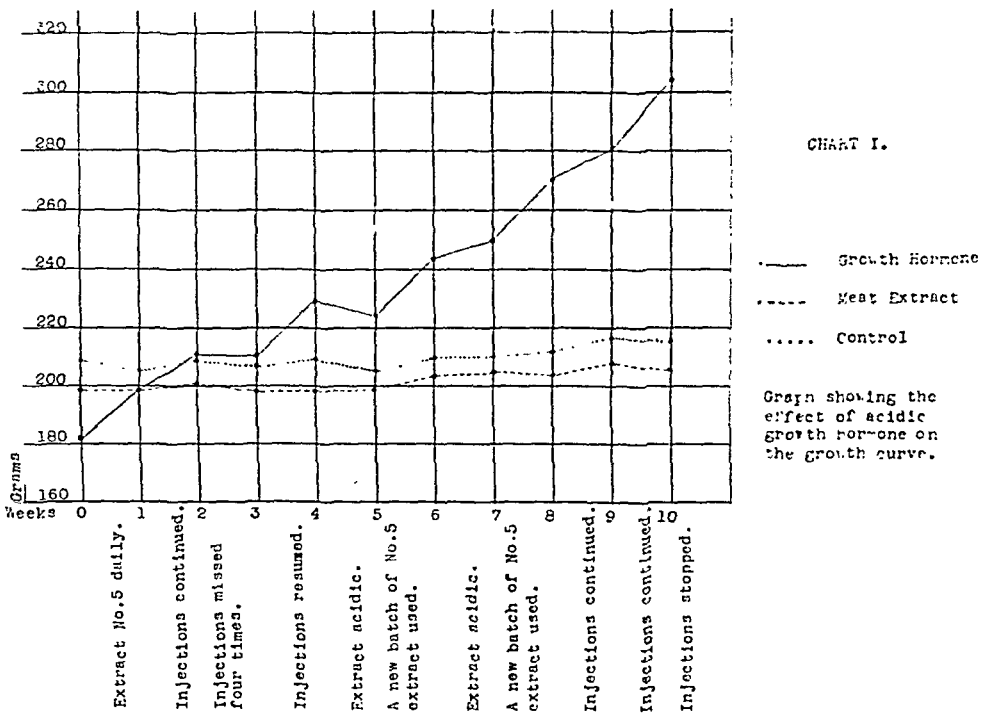


Chart I.

One of the animal groups which had reacted alternately by growth increase and weight loss was then subdivided into two. To one of these the acidified extract was continued; to the other, a fresh alkaline preparation was administered (Chart II).

It was noted that the animal receiving fresh extract again gained weight while the animal which was continued on acidified extract failed to show any gain. Some of the animals receiving acidified extract actually lost weight after a previous increase had been noted (Charts I and II). It was also found that the addition of sufficient alkali to restore the original P_H after acidity had occurred failed to restore the potency of the extract. This failure was to be expected, since if addition of alkali to acidified extract could restore

potency, the buffers of the blood should be sufficient to produce this alkalinity and growth would be obtained even after acidity had occurred.

In order to prevent the use of inactivated (acidic to phenolphthalein) extract, it was found advantageous to add an indicator to the growth hormone preparation. Accordingly, two drops of a 1 per cent alcoholic phenolphthalein solution were added to each 30 c.c. of the pituitary extract and preparations were used only as long as they retained a pinkish tinge.

Where an extract is made and immediately placed on ice, its potency lasts for several months, but where refrigeration is imperfect as in those instances where the extract has to be shipped it appears that an indicator is of value.

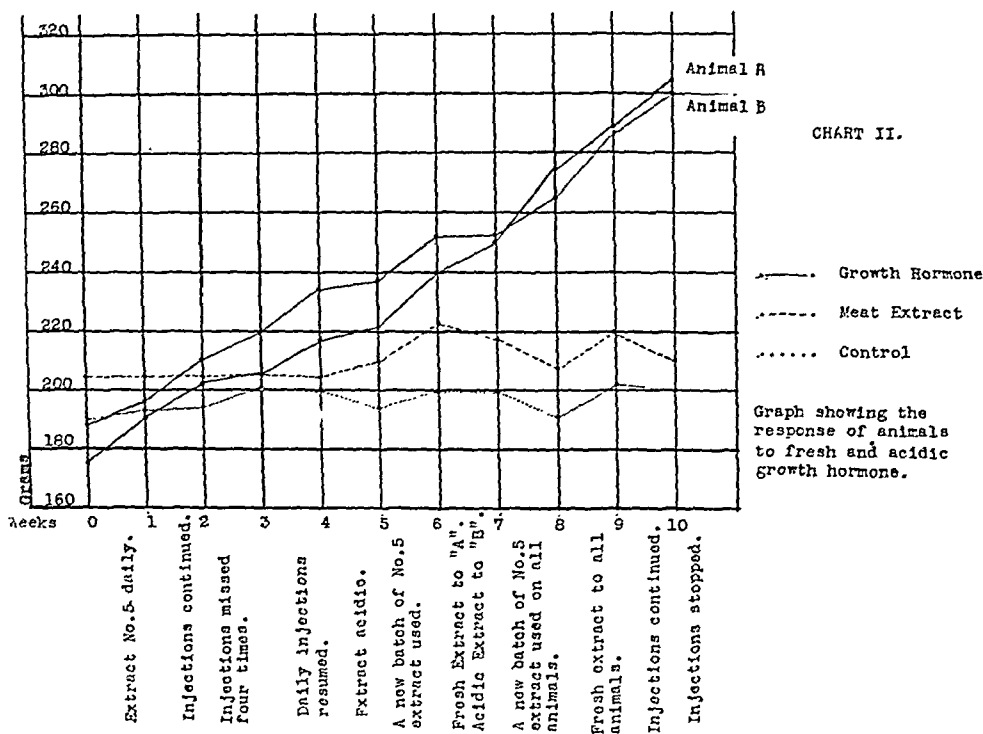


Chart II.

SUMMARY

1. Alkaline extracts of the anterior pituitary gland become less alkaline as a result of imperfect refrigeration.
2. This decreased alkalinity inactivates the growth hormone.
3. Adequate refrigeration decreases the rapidity of inactivation.
4. The adjustment of P_H after inactivation, fails to restore the potency of the extract.
5. Phenolphthalein added as an indicator serves as an index to the alkalinity of the preparation which must be maintained if the extract is to remain potent.

The author is greatly indebted to Dr. Carl L. Davis, head of this Department, at whose suggestion this paper was written.

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THE TREATMENT OF BRONCHOPULMONARY SUPPURATION OF FUSOSPIROCHETAL ETIOLOGY WITH SMALL DOSES OF NEOSALVARSAN*†

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FUSOSPIROCHETOSIS is gradually gaining recognition as an important factor in bronchopulmonary pathology. Receiving its first clinical recognition in Ceylon, India, in 1906, by Castellani,¹ who described it under the name of "bronchopulmonary spirochetosis," it has, through its cosmopolitan character, gained the attention of clinicians the world over.

It is with the hope of stimulating further study in regard to treatment of this deserving subject, that an attempt is made to relate the experiences gained from a study of 25 cases of acute and chronic bronchopulmonary suppuration in whose sputum, spirochetes, fusiform bacilli, vibrios, and other anaerobic organisms were repeatedly found.

To begin with, it should be stated that about 80 per cent of normal mouths and practically all pyorrheic mouths harbor anaerobic organisms, particularly spirochetes. These organisms are found about the teeth, tonsils, and adenoids, as pointed out by Davis and Pilot,² Carpenter,³ and others.

That the buccal anaerobes may produce serious diseases was called to our attention by Plant⁴ in 1894 and Vincent⁵ in 1896 when they pointed out the relationship of these organisms to Vincent's angina. That aspiration of oral contents into the lungs can easily take place has been demonstrated in animal experiments by Kline,⁶ Smith,⁷ Crowe and Scarff,⁸ Allen,⁹ Hedblom, Joannides and Rosenthal,¹⁰ Harkavy,¹¹ Schlueter and Weidlein,¹² Bethune,¹³ and clinically, by Myerson,¹⁴ Daily and Daily,¹⁵ Iglauer,¹⁶ and Ochsner.¹⁷

There has been considerable discussion as to whether or not the aspirated mouth organisms are merely saprophytic contaminants of the tracheobronchial tree or actual pathogens. There is a growing belief that a previous pyogenic infection may prepare the way for the action of the anaerobes in symbiosis and contribute to the pathogenicity of the lesion.

Predisposing factors of bronchopulmonary spirochetosis are poor hygiene of mouth, such as pyorrhea alveolaris, carious teeth, infected tonsils and ade-

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noids, infected sinuses, and Vincent's angina. Operations on the upper respiratory tract or operations in general under deep anesthesia may predispose to the development of the condition. General debilitating diseases such as diabetes, pulmonary tuberculosis and cancer may be associated with the condition. The existence of previous bronchopulmonary pathology such as chronic bronchitis and emphysema, bronchiectasis, and chronic fibrosis of the lungs in general, may predispose to the development of bronchopulmonary spirochetosis.

TREATMENT

From the preceding discussion it is evident that mouth hygiene is one of the indispensable measures in the prophylactic control of this condition. Carpenter³ states that patients without teeth and tonsils only occasionally harbor spirilla, and suggests the thought that to eradicate fusospirochetal diseases from the human race, we must remove all teeth, tonsils, and adenoids.

A review of the literature reveals the following general procedures available for the treatment of acute and chronic suppuration of the lung: (a) Prolonged bed rest, (b) bronchoscopy, (c) pneumothorax, (d) oleothorax, (e) phrenicoectomy, (f) incision and drainage, (g) thoracoplasty (partial or complete), (h) lobectomy and cauter pneumectomy, (i) heliotherapy, (j) transfusions, (k) vaccine therapy, and (l) medicinal measures.

A study of the literature reveals that many drugs have been recommended for the treatment of this condition, such as tartar emetic, iodine, antimony, emetine, bismuth, and the various arsenicals such as sulpharsphenamine, salvarsan, and neosalvarsan. Vincent¹⁸ also reported the use of a serum for the treatment of gangrene.

It is the purpose of this paper to limit the discussion to the value of the arsenicals in general and neoarsphenamine in particular in the treatment of suppuration of the lungs of fusospirochetal origin.

It is amazing to find that until very recently most writers actually failed to mention the arsenicals in their discussion of treatment of suppuration of the lungs.

Due to the accumulation of evidence strongly pointing to the potential pathogenicity of the mouth organisms, and due to the possibility that the various forms of acute and chronic suppuration of the lungs may merely represent different phases of the disease fusospirochetosis,¹⁹ more attention is being given to the drug. Some physicians believe in its specificity, others fail to see any value in its use. On the one hand, we find Kline and Berger²⁰ who state, "Arsphenamine therapy is apparently as specific in this as in other spirochetal infections." On the other hand there is Miller²¹ who states, "Specific therapy, particularly arsphenamine, is by no means regularly successful. In fact, in my experience, it rarely has proved to be of real specific value."

Why these opposing views, is a question that immediately confronts one and tempts one to speculate for a possible answer. It is interesting to note in this connection that to date one fails to find in the available literature any scientific discussion of dosages, frequency of administration, and reasons for favorable or unfavorable results in the treatment of suppuration with arseni-

cals. Some writers merely mention the drug without qualifying the dose; others content themselves with the statement that the dosages used are the same as in syphilis; while still others advocate intensive doses such as 0.6 to 0.9 of a gram of neosalvarsan.²⁰

The value of large doses of arsenicals in the treatment of suppuration was first questioned by me²² about three years ago when neosalvarsan was used by me in two cases of abscesses complicated by gangrene. The initial dose used in each patient was 0.3 gm. In each instance unfavorable reactions followed the administration of the drug; the temperature rose to a higher level, dyspnea was more marked, and the sputum became more foul. Both patients gradually became worse and died within a few days. The opportunity to investigate the possible value of small doses of neosalvarsan in the treatment of this condition did not present itself until October, 1930,

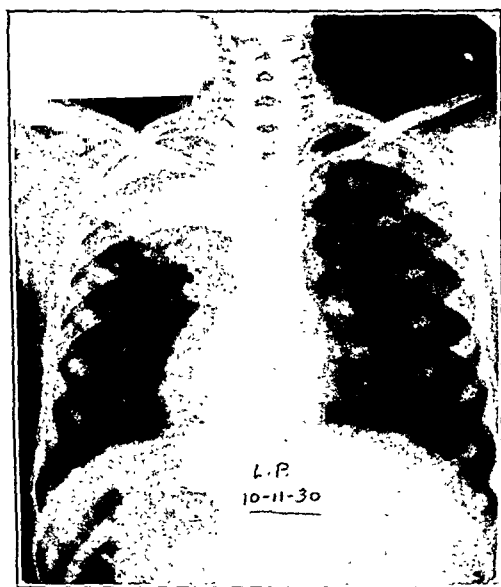


Fig. 1.

when three of the patients with acute suppuration of the lungs failed to show improvement, and were definitely worse after a reasonable time of treatment with conservative measures. However, before instituting radical treatment which seemed almost compelling, it was decided as a last conservative measure, to use neosalvarsan in small doses.

To evaluate properly the results obtained with this drug in small doses curtailed case histories of the three patients will be helpful.

CASE 1.—L. P., white boy, thirteen years of age, admitted to St. Mary's Infirmary (a part of St. Louis University group of hospitals) on Sept. 29, 1930, six days following the removal of his tonsils and adenoids under general anesthesia. On September 27 he had a sudden severe pain in left chest region, followed by fever, cough, and vomiting. On the day of admittance, the sputum became slightly foul. The white count was 8200, red count, 4,525,000, and hemoglobin 90 per cent. The temperature was 103°, pulse 120, and respirations 28. Sputum was negative for T.B., positive for elastic tissue, and sputum culture re-

vealed pneumococci, streptococci, *Micrococcus catarrhalis*, and a gram-negative rod. There was a marked shift to the left with the Arneht Schilling method.²³ The blood sedimentation rate²⁴ was rapid and the Wassermann was negative. Physical examination revealed dullness and diminution of breath sounds and inconstant posttussive clicks in region of left apex. A diagnosis of lung abscess was made which was confirmed by a roentgenogram (Fig. 1).

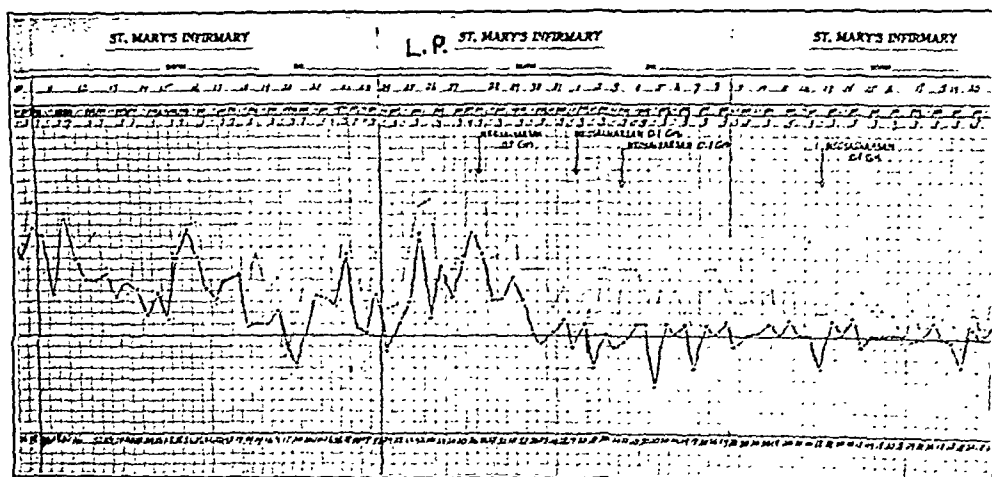


Fig. 2.

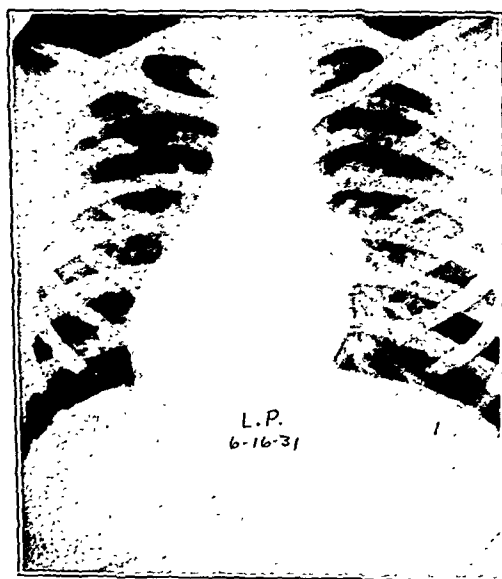


Fig. 3.

The patient was treated conservatively with bed rest, postural drainage, and symptomatic treatment. However, in spite of this treatment he became definitely worse. On the twelfth day after admittance, the sputum assumed a grayish greenish appearance, became definitely bloody, and very offensive in character. The temperature at this time was 104°, pulse 146, and respirations 48, and the white count rose from 20,100 on the twelfth of October to 33,800 on the sixteenth of October. At this time the patient was also having a hemorrhage. A diagnosis of beginning gangrene was made. On the twenty-seventh of October a dark-field examination of the sputum revealed numerous spirochetes. On the twenty-eighth of

October, four weeks after admittance, he was given 0.05 of a gram of neosalvarsan intravenously, and twenty-four hours afterward, there was a drop in fever to 101° and in forty-eight hours to 99° as seen in Fig. 2. With this drop in fever general improvement was noticed. The sputum diminished in quantity and was no longer foul. Patient was given 0.1 gm. of neosalvarsan on the first and fourth of November, 1930, and was discharged on

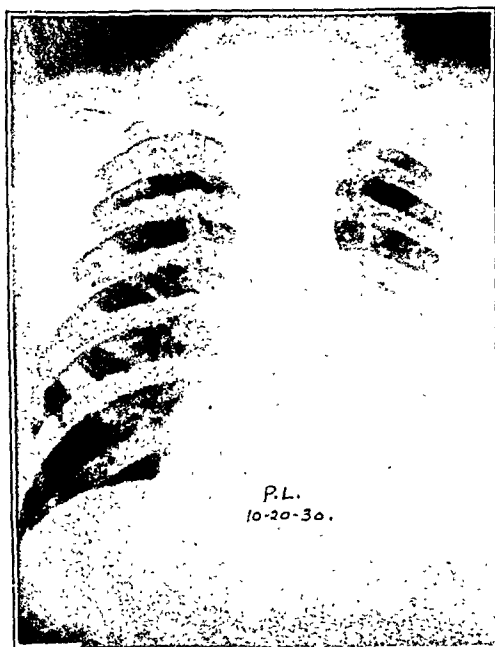


Fig. 4.

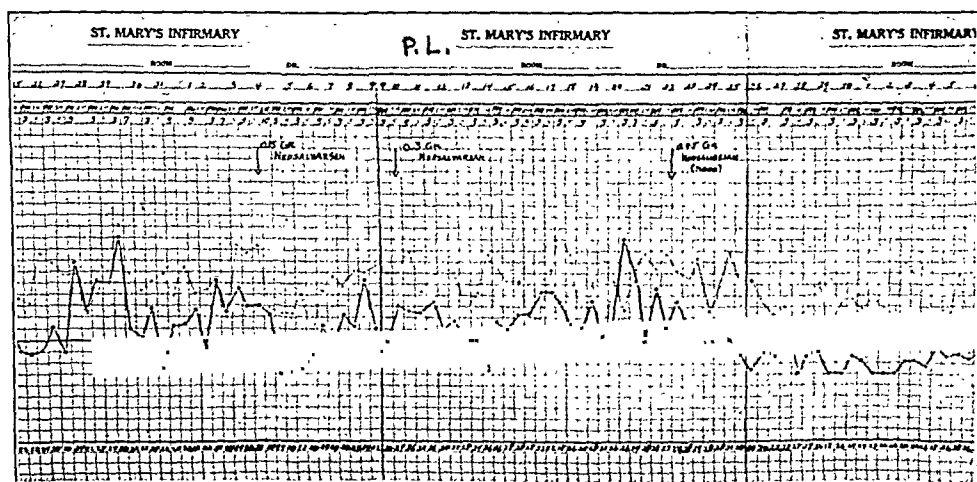


Fig. 5.

Jan. 2, 1931 very much improved. He was observed in the chest clinic until cured as seen in roentgenogram (Fig. 3). A reexamination on Jan. 7, 1932 revealed that the patient remained well.

CASE 2.—P. L., white male, aged twenty-six, admitted to hospital on Oct. 10, 1930 complaining of pain in right chest region, cough, loss of weight, tired feeling, fever, and

sweats, all these symptoms having been present since onset four weeks prior to his entrance into the hospital. He noticed no improvement while at home despite the fact that he was kept at complete bed rest. Temperature at entrance was 101° , pulse 120, respirations 24, W.B.C. 12,400, R.B.C. 4,830,000, hemoglobin 88 per cent. Arueth Schilling count showed a marked shift to the left. Sedimentation rate was rapid. Nonprotein nitrogen 29, blood sugar 98, Wassermann negative, urine negative, sputum negative for T.B., but revealed streptococci, pneumococci, *Micrococcus catarrhalis*, and gram-negative rods. Physical examination revealed definite limitation of motion on the right side, dullness and diminution of breath sounds and scattered indefinite râles from the fourth vertebral spine to base of right lung posteriorly. A roentgenogram (Fig. 4) showed an increase in density at base of right lung. A diagnosis of suppurative pneumonitis was made.

Patient was at first treated conservatively, but no improvement was noted after twenty-five days of such treatment. The afternoon temperature varied from 101° to 102° , pulse 100 to 120, and respirations 24 to 30. A roentgenogram showed a definite extension of

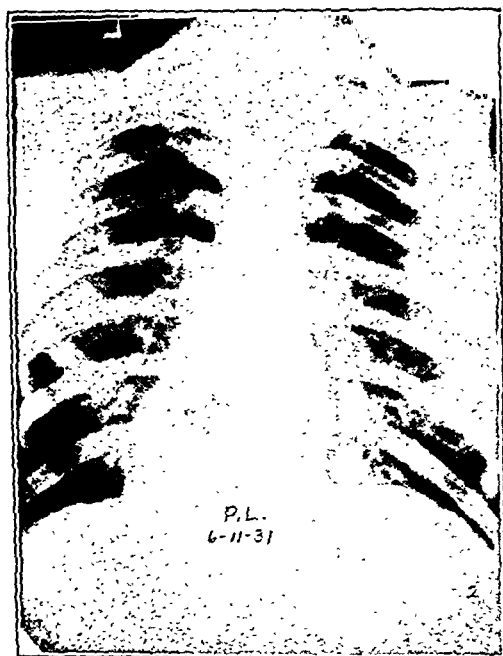


Fig. 6.

the process. Repeated dark-field examinations of sputum at this time revealed fusiform bacilli. On Nov. 5, 1930, twenty-five days after admittance, he was given 0.15 gm. neosalvarsan and within twenty-five hours, subjective improvement was noticed and also a temporary drop in the fever to 99.6° . Three-tenths grams of neosalvarsan was then given on Nov. 9, 1930, after which time the temperature again dropped temporarily to 100° as seen in Fig. 5. On November 22, thirteen days after, the second dose, 0.45 gm. of neosalvarsan was given after which time the temperature dropped to normal and the patient improved steadily to complete recovery as seen in Fig. 6.

CASE 3.—R. D., white female, aged twenty-seven, admitted to hospital on Nov. 3, 1930 with the following complaints: Weakness, loss of strength, pains in chest and lumbar region, chills and fever, shortness of breath. She was delivered of a baby two months prior to her entrance. A month following delivery she began to have chills followed by fever. Two weeks before entrance she began to have daily chills. Her physician diagnosed her condition as malaria, and she was treated for this condition. Failing to improve, she entered the hospital. Temperature varied from 97° in A.M. to 105.8° in P.M., pulse, from 90 to 130,

and respirations, from 20 to 28. W.B.C. 11,500, R.B.C. 3,470,000. Hemoglobin 85 per cent. Urine (noncatheterized) specimen showed 2-plus albumin, and a few pus cells. Catheterized specimen showed urine to be negative. Urine culture was negative. A Schilling count showed

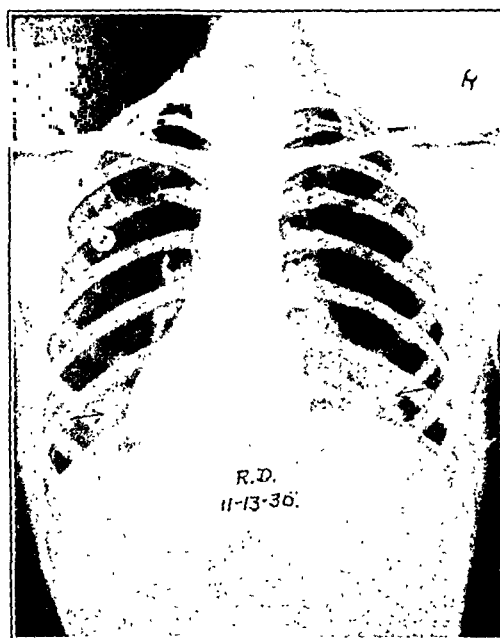


Fig. 7.

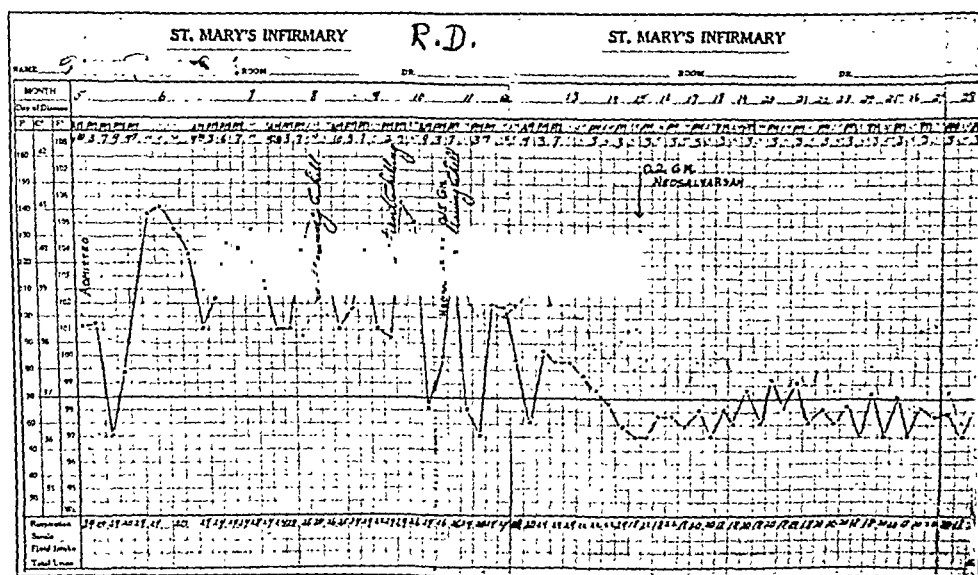


Fig. 8.

a shift to left and was negative for malarial parasites. Wassermann was negative. Non-protein nitrogen was 29. Blood sugar was 91. Blood culture was negative. Stools revealed a yeastlike organism. Sputum showed numerous spirochetes and fusiform bacilli with the carbolfuchsin stain. Physical examination revealed the essential pathology to be in the

chest as follows: Dullness on percussion in lower one-third of both lungs, but especially on the left side. Harsh breath sounds in lower half of both lungs especially pronounced on left side. Scattered fine posttussive râles in lower half of both lungs. Bronchial breathing opposite sixth vertebral spine on the left side. Gynecologic examination by the gynecologic department failed to reveal any pathology in the pelvis to explain the chills and fever. A roentgenogram (Fig. 7) revealed a small area of density at base of left lung close to heart



Fig. 9.—Anaerobes from a spirochetel empyema.

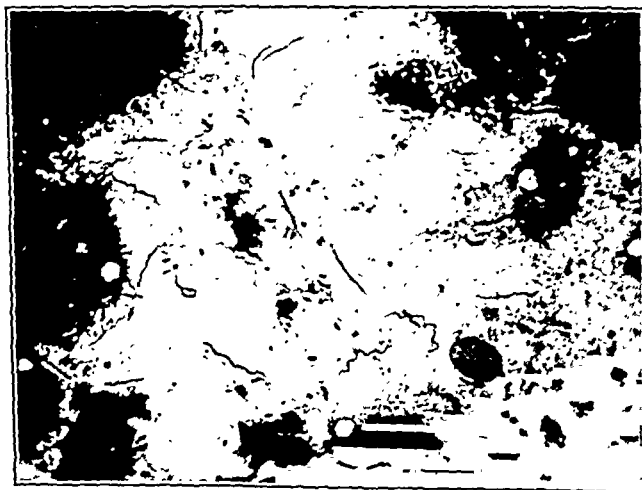


Fig. 10.—Anaerobes from the sputum of a case of spirochetel abscess.

and one suspicious shadow in second interspace. A diagnosis of multiple lung abscesses seemed to be justifiable under the circumstances.

Patient was treated for possible pyelitis with no results. Neosalvarsan was given 0.15 gm. on November 10, and temperature dropped to 102° the next day; the chills did not recur. In forty-eight hours the temperature dropped to 100°. Two-tenths gram was given on November 15, five days after first dose, and temperature dropped to normal the next day and remained normal as seen in Fig. 8. Clinical improvement was very rapid. Patient made a complete recovery later. In answer to a follow-up letter that was sent to her on Jan. 2, 1932, she stated that she had remained well.

to large doses. The report of the Salvarsan Council of Great Britain³⁰ and the recent article by Cole³¹ and coworkers definitely points to the fact that accidents in the treatment of syphilis are most common when large doses are used.

The most important proof in favor of small doses, however, is the experimental work of Toyama and Kolmer³² who have shown that large doses of either arsphenamine or mercury directly interfere with the nonspecific defense, and diminish the production of agglutinins and lysins with which the body carries on its own defense against the invader. On the other hand, minute doses have a definitely stimulating effect on the patient and seem to encourage the formation of agglutinins and lysins.

In summarizing the possible action of small doses of neosalvarsan in the treatment of fusospirochetosis, it is conceivable that the salvarsan, through its specific spirillicidal action, kills the spirochetes, and through its nonspecific tonic action stimulates the formation of agglutinins and lysins which in turn destroy the other members of the symbiotic group.

I am greatly indebted to Dr. G. O. Broun, Dr. R. Kinsella and Miss E. McGarry for their assistance and encouragement in this work.

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UNIVERSITY CLUB BUILDING

THE GROWTH OF PATHOGENIC BACTERIA IN IMMUNE BLOOD*

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WHEN one consults a text on the cultivation of pathogenic microorganisms from the blood stream, it is generally stated that the procedure consists of withdrawing from 10 to 20 c.c. of blood from a vein and injecting this into 50 to 100 c.c. of broth culture medium, and that this large quantity of culture medium is necessary to dilute the antibodies in the serum, the antibodies being bactericidal. The directions, also, generally state that 5 c.c. of blood may be added to 10 c.c. of melted agar culture medium and plated. Other procedures may call for the addition of the blood to citrated or bile culture medium, the addition of these agents to the broth culture being to destroy the bactericidal action of the blood, particularly the serum. Stitt¹ gives the following technic: "As a receptacle for the blood we employ Erlenmeyer flasks of 100 c.c. capacity, containing 10 to 25 c.c. of salt solution with 1 or 2 per cent of sodium citrate, for prevention of coagulation. In this way we may take from 10 to 25 c.c. of blood. For plates add 2 or 3 c.c. of this citrated blood to 6 or 8 c.c. of melted agar at 45° C. Finally we place the receiving flask in the incubator and culture it as well as the other media. Some organisms do not develop in this citrated blood, possibly on account of the blood concentration. This can be avoided by adding 50 to 75 c.c. of bouillon to the citrated blood remaining in the flask. For culturing blood in septicemic conditions use at least ten times as much bouillon as blood."

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Muir and Ritchie² state: "In making blood cultures in order to detect the presence of enteric bacilli 5 c.c. of blood are added to 10 c.c. of sterilized ox bile or 50 c.c. of a 0.5 per cent solution of bile salts in 1 per cent sodium citrate. In examining for other organisms, e.g., streptococci, 5 or 10 c.c. of blood are added to a large quantity of fluid medium (100 c.c. or more)."

Clawson³ draws 50 c.c. of blood and distributes this in two tubes and allows the blood to clot. The serum is withdrawn and the clot removed to 250 c.c. of dextrose beef infusion broth. He states that the firm clot seems to favor a partial anaerobic culture, and at the same time any antibodies that may be present are diluted in the fluid medium.

Cecil, et al.⁴ withdraw 20 c.c. of blood and divide into two tubes. After the clot has formed the serum is withdrawn, the clot broken and transferred to 50 c.c. of beef heart infusion broth. This procedure is used to rid the culture of antibodies.

Mesnil⁵ reported, in 1898, that the serum of rabbits which had been immunized against *Bacterium erysipclatis suum* makes an excellent medium for the culture of this microorganism.

Bridré and Jouan⁶ showed that the observation of Mesnil was also true in regard to horses, and that the *Bact. erysipclatis suum* grew better in the immune serum than in the serum of normal horses. These authors found that the growth in the bouillon which contained the immune serum (10 to 20 per cent) was about six times as great as that containing an equal amount of normal horse serum.

This phenomenon was studied by Nicolle and Césari.⁷ These authors found that the best culture medium was one made of Martin's bouillon 9 parts and immune serum one part. The organisms studied were anthrax, tetanus, gonococcus, diphtheria, typhosus, paratyphosus A, and pneumococcus Types I, II and III. All of these bacteria grew more abundantly in the medium containing the homologous immune serum. The growths of the gonococcus and the pneumococcus were not as readily apparent as the growths of the other microbes, but there was some better growth in the medium containing the immune serum. Some of the organisms grew so abundantly as to form a scum on the surface of the medium; such does not occur in the ordinary culture media.

The phenomenon that concentrated immune serum is not bactericidal in vitro has been known for years. This is called the "phenomenon of Neisser and Wechsberg." These authors found that there was no bactericidal action on the typhoid bacillus when the typhoid immune serum was added to an equal amount of a typhoid culture, but that there was a bactericidal effect when the immune serum was diluted, and that this effect was lost when the dilution reached a certain figure.

Relative to this phenomenon Muir and Ritchie,² page 209, state: "It is to be noted that in the case of a bactericidal serum there is an optimum amount of immune-body which gives the greatest bactericidal effect with a given amount of complement. If this amount of immune-body be exceeded, the bactericidal action becomes diminished and may be practically annulled. This

result, which is generally known as the 'Neisser-Wechsberg phenomenon,' has been the subject of much controversy, and cannot yet be said to be satisfactorily explained; it is apparently of the nature of a 'zone phenomenon.' "

Bolton⁸ found that normal serum may kill relatively more bacteria when diluted than when undiluted.

Neisser and Wechsberg⁹ state that if the immune serum be greatly diluted its action is changed and it loses its general bactericidal power and develops a marked increase in the destructive action upon the homologous organism.

EXPERIMENTAL

In order to test out some of the above statements rabbits were immunized with *B. typhosus*, *B. abortus*, and a streptococcus. The titers of these sera were 1:6400, 1:3200, and 1:100 respectively. The sterile sera were placed in sterile tubes and inoculated with the homologous organism. The growth was profuse in all of the cultures. Blood was also withdrawn from each rabbit and defibrinated by the use of a sterile Erlenmeyer flask which contained broken glass; a separate flask was used for each rabbit. Each defibrinated blood was inoculated with its homologous organism. The organisms grew abundantly in these media.

The blood serum of a patient having a titer of 1:25000 for *B. typhosus* was available, at this time, as was the organism isolated from the patient. The undiluted sterile serum was inoculated with the homologous organism. The organism grew so abundantly that it formed a scum on the surface of the medium. The growth was so rank in the three tubes which were used that it was at first thought that the serum was contaminated. This experiment was repeated with other strains of *B. typhosus* and the same phenomenon occurred. Other cases of typhoid fever became available as time went on and this same procedure was followed with the same results.

It was decided to apply the above facts to the isolation of microorganisms from the blood stream, i.e., blood cultures. The technic consisted of withdrawing 20 c.c. of blood and discharging 5 c.c. into each of two culture flasks containing 50 c.c. of veal heart infusion broth, and the remaining 10 c.c. were discharged into an Erlenmeyer flask of 50 c.c. capacity containing broken glass (glass beads may be used) to defibrinate the blood. In several experiments a 100 c.c. flask was used and 20 c.c. of blood placed in the flask. The flask containing the defibrinated blood was placed in the thermostat like any other culture. The flasks were examined and transplants made as usual.

It is apparent that there are but a given number of bacteria per cubic centimeter of blood in a blood stream infection, and in carrying out an investigation with standard methods for controls it is possible that the bacteria may be present only in the blood discharged into the defibrinating flask; it is likewise possible for the organism to be discharged from the syringe only into one of the flasks containing the broth; however, no culture, to date, has been obtained in the broth and not in the defibrinated blood, while the reverse has occurred on a number of occasions.

The following organisms have been isolated from the blood stream by the defibrinated blood technic: *B. typhosus*, *B. paratyphosus* B, *B. abortus*, gonococcus, *M. catarrhalis*, and streptococcus.

COMMENT

The literature on the methods of taking blood cultures assumes that whole blood is bactericidal, due to the serum. The work of Neisser and Wechsberg shows that this is not the case. This is further substantiated by the work of Nicolle and Césari who found that the immune serum stimulated the growth of the homologous organism. It appears to me that it is but logical to assume that such would be the case, inasmuch as the infecting agent is living in the blood stream, or at least in the tissues served by the blood, and that in vivo, at least, this makes a most excellent medium; a better medium, perhaps, than the blood of a normal animal, or individual in the case of human beings. The above experiments seem to bear out this assumption.

The work of some investigators would indicate that the bactericidal zone of the serum is that in which most of the cultures are diluted in the standard methods. Due to the number of positive cultures obtained in this zone, it appears that the bactericidal zone varies widely.

The use of defibrinating flasks for blood cultures simplifies the taking of these cultures and gives a satisfactory medium for the infecting organism. The defibrinated blood may be covered with a layer of oil for the cultivation of anaerobes, although, experimentally, anaerobes were grown in the defibrinated blood without oil by using a small flask and withdrawing an extra amount of blood to give extra depth of medium for anaerobic conditions.

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LABORATORY METHODS

AN AUTOMATIC AND GRAPHIC METHOD OF RECORDING DIURESIS*

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IN CONNECTION with experiments on the relationship of diuresis to recovery from barbital poisoning reported elsewhere by us,¹ we looked about for a suitable method for recording the rate of urine flow from barbitalized dogs over an extended portion of the recovery period. The nature of the experiments was such that it was necessary to know whether a given urine volume collected over an eight- to twenty-four-hour period represented uniform renal

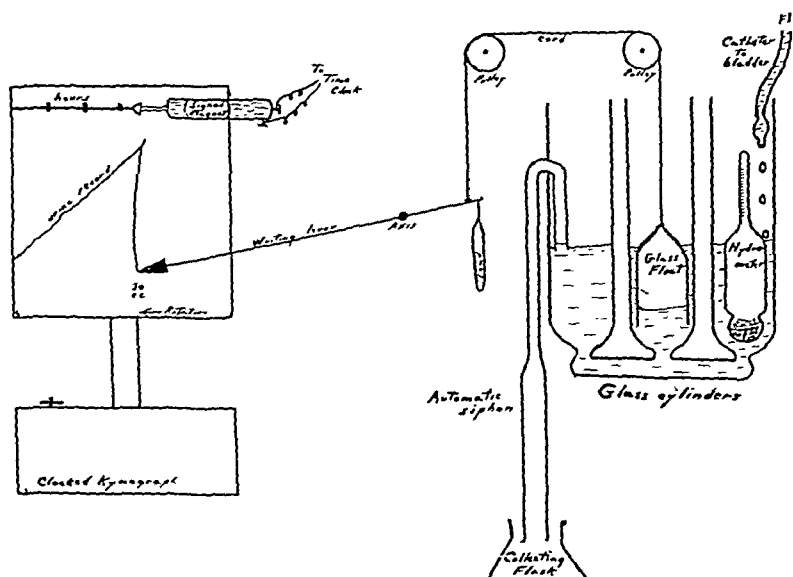


Fig. 1.—Apparatus for recording the rate of urine output. For explanation see text.

performance over the entire period or an increasing, decreasing, or fluctuating rate of flow. To suit our needs the record should be condensed, graphic, continuous, and automatic to the extent of not requiring constant or even exactly periodic attention on the part of the laboratory staff, and should permit fairly accurate calculation of the rate of flow for any increment of time. None of the existing devices with which we became familiar answered these requirements and the success of the method to be described was sufficient, perhaps, to warrant description.

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The female dogs used in these experiments were prepared by perineorrhaphy so that the urethra was easily accessible for catheterization. In most of the experiments barbitol anesthesia was obtained before the records were started, but a few records were made in nonanesthetized animals trained to lie quietly and submit to the administration of fluids and diuretic or anti-

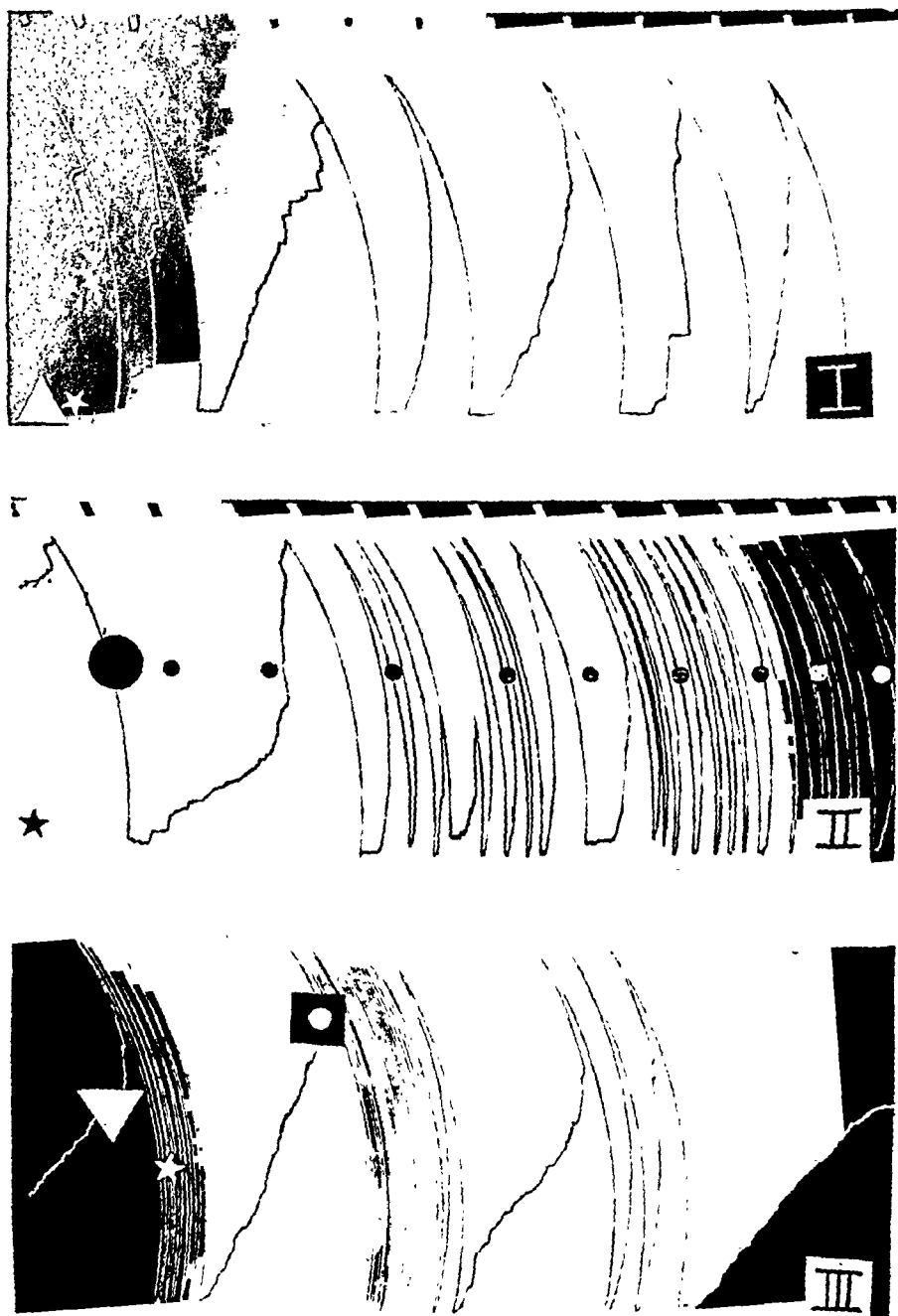


Fig. 2. (See legend on opposite page.)

diuretic principles. Under the experimental conditions the rates of urine flow varied from less than 2 to over 60 c.c. of urine per kilogram per hour in animals weighing from 5 to 20 kg.

In principle, the method consists of collecting the urine through a retention catheter adjusted in the bladder into a cylinder containing a float, the rise of which is recorded by means of a suitable lever system and writing point on a smoked drum moving about $2\frac{1}{2}$ cm. per hour. At the top of the excursion the cylinder is automatically emptied, returning the writing point to the base line for another trip.

The method allows of considerable variation and refinement. Our arrangement is diagrammed in Fig. 1, showing three upright parallel cylinders with communication at their bases, one for receiving the urine, one for operating the float, and a third for emptying the apparatus intermittently by siphon. Each excursion represents approximately 35 c.c. of urine. For clearness of illustration it is represented as being an all glass one piece apparatus; actually it was assembled by means of T-tubes, rubber tubing and glassware as found in the average laboratory.

In the use of the apparatus the greatest technical difficulty encountered is in the adjustment of the siphon to empty the cylinders with uniform excursions and urine volumes, and an electromechanical arrangement may eventually prove more satisfactory. The tendency for a few drops of liquid to become dispersed along the siphon tube and by their weight cause premature emptying, is reduced by extending the siphon tube with a larger glass or rubber extension to a flask several feet below the level of the table or on the floor. This extension accelerates the emptying process by the greater pull of the longer liquid column, draws fewer interspersed drops of fluid into the constricted upper portion of the siphon tube, and those lodging in the expanded portion drain down the walls of the tube establishing an air column without tendency to start the emptying mechanism. Before each experiment we have found it best to adjust the writing point to the newly smoked drum, and by introducing water into the apparatus, produce several satisfactory excursions before starting the record.

Three typical records of urine flow are shown in Fig. 2. These experiments are discussed in the paper previously referred to. Occasional right angle steps in the urine records are artefacts due to imperfect adjustment of

Fig. 2.—Graphic records of urine flow recorded by apparatus shown in Fig. 1. Weight of animal: 11.9 kilograms. Dose of sodium barbital: 4.0 grams given intravenously in 10 per cent aqueous solution on each occasion.

KEY: I. Record of urine flow from 10:45 A.M. to 10 P.M. Apr. 10, 1931.

△ 500 c.c. 0.9 per cent sodium chloride solution by stomach tube.

* Injection of sodium barbital.

II. Record of urine flow from 11 A.M. Apr. 17, 1931 to 12:30 A.M. Apr. 18, 1931.

* Injection of sodium barbital.

○ Intravenous drop injection of 1000 c.c. of 10 per cent glucose started.

o Completion of 1000 c.c. increments of above.

III. Record of urine flow from 9:45 A.M. Mar. 27, 1931, to 1:30 A.M. Mar. 28, 1931. Record started in unanesthetized animal.

▽ 500 c.c. of water given by stomach tube.

* Injection of sodium barbital.

□ Intravenous injection of 1000 c.c. of 10 per cent glucose in fifteen minutes.

the level of the catheter in the bladder and may be eliminated by the use of the expanded retention catheter used in bladder surgery.

We wish to acknowledge the aid of Dr. A. J. Carlson in arranging this material for publication.

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A STUDY OF THE EAGLE ANTIGENS FOR WASSERMANN AND FLOCCULATION TESTS*

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THE history of the serology of syphilis is rich in its variety of antigens. Ever since the introduction of an alcoholic extract of normal tissues¹ as an antigen for complement fixation in syphilis, numerous variations of this basic extract have been suggested to produce a more satisfactory and more sensitive antigen. If one could disregard the possibility of false positive reactions due to its intense anticomplementary action, a highly sensitive antigen could be prepared with comparative ease. To insure a margin of safety from false positive fixations, it has been our practice to use only antigens whose antigenic units (the minimum amount of antigen which will produce complete inhibition of hemolysis in the presence of known four-plus sera) are not more than $\frac{1}{100}$ of the anticomplementary unit for that antigen (minimum amount of antigen producing inhibition of hemolysis in the absence of positive sera).

As a routine antigen for the Wassermann test, we use the Kolmer antigen prepared from a cholesterinized alcoholic extract of powdered beef heart.² Each specimen of blood serum submitted to our laboratories for serum diagnosis of syphilis is tested by both Kahn and Kline precipitin methods in addition to the Wassermann test. Although we occasionally find a serum which is positive in a Wassermann test and negative in the precipitin test, generally speaking the precipitin reactions appear more sensitive than the Wassermann reaction. The hemolytic system for the Wassermann test is adjusted to maximum sensitivity and a margin of elasticity allowed for by using two units of complement and two units of amboceptor. These units are determined by graded titrations of the two reagents, using as a unit the smallest amount producing complete hemolysis after one-half hour at 37.5° C.

In November, 1930, Eagle of Johns Hopkins University Laboratories published a paper³ explaining the fortifying effect of cholesterol upon antigens

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used in serum diagnosis of syphilis. Subsequent to this (May, 1931) he published another paper¹ describing a more sensitive antigen for use in the Wassermann reaction. In May, 1932, the same author published a paper² describing the new flocculation test for syphilis.

In our laboratory, under the guidance of the senior author, there was made a study of the comparative values of the Eagle and Kolmer Wassermann antigens, and the Eagle, Kahn, and Kline precipitin tests.

The Kolmer antigen for Wassermann tests is somewhat tedious to prepare and not infrequently freshly prepared Kolmer antigens must be discarded because of insufficient antigenic property or too great anticomplementary activity. Eagle's Wassermann antigen is quite simple to prepare, and to date the authors have had no unsatisfactory lots of antigen prepared by this method.⁴ The increased sensitivity of this antigen is dependent on a high concentration of antigen-lipoids reinforced by 0.8 per cent cholesterolin and 0.6 per cent sitosterol.

Wassermann Technic.—To 0.1 c.c. of freshly inactivated blood serum is added 0.4 c.c. of Reagent A (ten units of antigen and two units of complement in 0.9 per cent NaCl). To a second tube containing 0.1 c.c. of the same serum is added 0.4 c.c. of Reagent B (two units of complement in 0.9 per cent NaCl). Control tubes containing 0.1 c.c. of 0.9 per cent NaCl and 0.4 c.c. of Reagents A and B respectively are placed in the rack with the sera to be tested. These are incubated for two hours at 8° C. To each tube is added 0.5 c.c. of 2 per cent sensitized cells (sensitized immediately before use) containing two units of amboceptor. The tests are then incubated in the water-bath at 37.5° C. until the two reagent controls are completely hemolysed and are left in the water-bath for ten additional minutes before reading. Although Eagle recommends the use of 0.2 c.c. of a 1-200 dilution of antigen without titration, in accordance with our customary procedure we titrated the antigen and used ten antigenic units as a dose.

From our serologic work we tested 1081 cases taken at random, giving no consideration to the case histories before testing. Each of these was tested by Kline's precipitin test, by Kahn's precipitin test (using one quantity of antigen only, 0.025 c.c.) and by Wassermann tests using Kolmer's and Eagle's antigens respectively, and the technic described above. Of the 1081 cases tested, 458 were positive by Kline's test, 346 were positive by Wassermann employing Eagle's antigen, 325 were positive by Kahn's test and 266 were positive by the Wassermann employing Kolmer's antigen. There were no positive K.A.W. (Kolmer antigen Wassermanns) unaccompanied by other positive serology (Kahn, Kline, or Eagle). Likewise there was only one positive Kahn in the absence of other positive serology; this was a treatment check case. Of the Klines, there were 66 positives with no other positive serology. Of these 58 were one-plus (one-plus reactions in any one test unaccompanied by other positive serology are not customarily reported by our laboratory), and 8 were higher positives. Their case histories were as shown in Table I.

TABLE I

	DIAGNOSIS	TREATMENT CHECK	NO HISTORY ON CARD
3-plus	1	1	1
2-plus	0	2	3
1-plus	25	9	24

There were 12 E.A.W. (Eagle antigen Wassermanns) which gave a one-plus reading in the absence of any other positive serology, and 9 higher positives distributed as shown in Table II.

TABLE II

	DIAGNOSIS	TREATMENT CHECK	NO HISTORY ON CARD
4-plus	0	1	1
3-plus	1	1	0
2-plus	3	1	1
1-plus	6	3	3

These results seemed to indicate a sensitivity of the E.A.W. approximately 1.3 times that of the K.A.W., with only a small percentage (21 out of 1081 tests) pointing to possible false positives. There were 325 E.A.W. accompanied by other positive serology, and 324 Kahn tests accompanied by other positive serology. The Kline test was somewhat more sensitive, having 400 positives accompanied by other positive serology. The specificity of these tests could not accurately be estimated by the above method of case selection, due to the fact that many of these cases were only submitted for a single examination and in most cases accompanied by very inadequate history. We therefore selected a number of known syphilitics from the skin clinics of the Indianapolis City Hospital and Fort Benjamin Harrison Hospital.

The ideal type of case would have been those showing early primary lesions, but these cases present themselves very infrequently. From the group of patients in the City Hospital clinics, we were able to secure only three primary cases. One of these, C.P., had been dark-field positive and serologically negative. After three treatments we tested his blood with the following results: K.A.W. four-plus, E.A.W. four-plus, Kahn negative, and Kline negative. A recheck four days later showed K.A.W. two-plus, E.A.W. three-plus, Kahn negative, and Kline negative. This was one of the rare cases in which there appears a greater affinity for the complement fixation phenomena than for the flocculation phenomena. A second case was that of G.A., dark-field positive and with the following serologic findings: K.A.W. negative, Kahn negative, Kline negative, and E.A.W. three-plus. A quantitative E.A.W. (0.1 c.c., 0.05 c.c., 0.025 c.c., and 0.005 c.c. respectively) read three-plus, three-plus, two-plus, and two-plus. A third patient, C.S., having a positive dark-field had the following serology: K.A.W. two-plus, E.A.W. two-plus, Kahn three-plus, and Kline two-plus.

TABLE III

PATIENT	NUMBER OF TEST	K.A.W.	E.A.W.	KAHN	KLINE
1. L.B.	1	Neg.	3-plus	2-plus	2-plus
	14 day interval				
	2	Neg.	4-plus	Neg.	4-plus
2. C.C.	1	1-plus	2-plus	2-plus	Neg.
	14 day interval				
	2	2-plus	4-plus	2-plus	4-plus
3. H.C.	1	Neg.	Neg.	Neg.	2-plus
4. W.C.	1	Neg.	2-plus	Neg.	Plus-minus
5. C.D.	1	1-plus	3-plus	4-plus	2-plus
	17 day interval				
	2	Neg.	1-plus	Neg.	Neg.
6. M.F.	1	Neg.	2-plus	3-plus	4-plus
7. T.G.	1	Neg.	Neg.	Neg.	Neg.
	12 day interval				
	2	Neg.	Neg.	Neg.	Neg.
8. H.G.	1	Neg.	Neg.	Neg.	Neg.
	12 day interval				
	2	Neg.	Neg.	Neg.	Neg.
9. R.G.	1	Neg.	Neg.	Neg.	Neg.
	12 day interval				
	2	Neg.	Neg.	Neg.	Neg.
10. A.G.	1	Neg.	Neg.	Neg.	Neg.
11. R.H.	1	1-plus	4-plus	Neg.	2-plus
12. A.H.	1	Neg.	4-plus	3-plus	2-plus
13. E.H.	1	Neg.	4-plus	4-plus	4-plus
14. O.H.	1	Neg.	2-plus	Neg.	2-plus
15. B.L.	1	Neg.	4-plus	4-plus	4-plus
16. M.L.	1	Neg.	3-plus	2-plus	4-plus
	2 day interval				
	2	Neg.	Neg.	2-plus	4-plus
17. P.M.	1	Neg.	1-plus	Neg.	1-plus
18. A.M.	1	Neg.	Neg.	Neg.	Neg.
19. G.N.	1	Neg.	Neg.	Neg.	Neg.
20. B.N.	1	Neg.	Neg.	Neg.	Neg.
21. M.P.	1	Neg.	1-plus	Neg.	Plus-minus
	2 day interval				
	2	Neg.	Neg.	Neg.	Plus-minus
22. W.R.	1	Neg.	1-plus	Neg.	1-plus
23. L.R.	1	Neg.	4-plus	4-plus	4-plus
24. E.R.	1	3-plus	4-plus	Neg.	2-plus
25. S.R.	1	Neg.	Neg.	Neg.	Neg.
26. D.S.	1	Neg.	4-plus	Neg.	Neg.
	14 day interval				
	2	4-plus	4-plus	2-plus	4-plus
27. J.S.	1	Neg.	1-plus	Neg.	4-plus
28. P.S.	1	Neg.	Neg.	Neg.	Neg.
	7 day interval				
	2	Neg.	Neg.	Neg.	Neg.
29. R.S.	1	Neg.	Neg.	Neg.	Neg.
30. J.S.	1	Neg.	4-plus	4-plus	4-plus
	7 day interval				
	2	Neg.	Neg.	4-plus	3-plus
31. E.T.	1	Neg.	Neg.	Q.N.S.	Neg.
	11 day interval				
	2	Neg.	Neg.	Neg.	Neg.
32. B.T.	1	Neg.	3-plus	Neg.	4-plus
	11 day interval				
	2	Neg.	3-plus	4-plus	1-plus
33. O.T.	1	Neg.	3-plus	Neg.	2-plus
34. M.Y.	1	1-plus	4-plus	4-plus	2-plus
35. J.W.	1	Neg.	Neg.	Neg.	Neg.
36. Z.W.	1	1-plus	4-plus	4-plus	4-plus

We tested four cases of primary syphilis from Fort Benjamin Harrison. All four had been diagnosed on positive dark-fields and immediate treatment instituted. Two separate testings twelve days apart revealed no positive serology.

Thirty-six "treatment check" cases were tested serologically. The results are tabulated in Table III.

The above results show a close coincidence between the E.A.W. and the precipitin tests. The figures in Table III show a sensitivity of the E.A.W. 3.5 times as great as that of K.A.W. Cases 5 and 26 are the only incidences of a positive E.A.W. in the absence of all other positive serology. The latter developed positives in all three other tests after an interval of fourteen days. The senior author had suggested the possibility that the antigen might prove so sensitive that it would hold a positive indefinitely in treated cases. It was with this possibility in mind that the above series of treated cases was tested, and from the results of this series, we believe we can state that such is not the case. As a further check on this point we ran all four tests on 74 treatment check cases showing completely negative serology by our routine tests. Two of these gave a 1-plus E.A.W., one a 2-plus, and one a 4-plus E.A.W. The others were negative by this test also. Twenty normal individuals were tested by all four serologic procedures and were all negative in every test.

CONCLUSIONS

After having submitted the Eagle Wassermann antigen to the test routine described above, we are convinced that it is a more sensitive antigen than the Kolmer antigen, and that tests in which this antigen is employed correspond very closely to the Kahn and Kline precipitin tests in degree of sensitivity. We believe that in this series of tests it has not proved too sensitive.

THE EAGLE FLOCCULATION TEST

An antigen for this test was prepared according to Dr. Eagle's directions⁵ and one thousand sera treated in the manner he described; K.A.W., Kahn, and Kline tests were done on each specimen. Strong positives and negatives could be very easily and accurately read by this test, but the intermediate zone, which Dr. Eagle characterizes as "doubtful," we were unable to read satisfactorily. Accurate readings of low positives were certainly no easier for us than in the case of a one-plus or two-plus Kahn test. We found in the case of strongly positive sera that this test checked very closely with the other serologic tests. Cases showing a negative Wassermann test and two-plus or three-plus Kahn and Kline were rarely positive by the Eagle Flocculation method. The method is more time-consuming than either the Kahn or Kline method for precipitin tests. Therefore, with the exception of this brief survey, no further work was done with this test.

We acknowledge with gratitude the cooperation of the clinicians of the Indianapolis City Hospital Skin Clinic and the Medical Staff of Fort Benjamin Harrison.

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AN EASILY ASSEMBLED, SELF-RECORDING PERFUSION APPARATUS*

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THIS apparatus was devised for the study of the volume of blood flow through the cat's carotid at fixed pressures over long periods of time, but may prove useful for other types of perfusion.

The fundamental mechanism employed is the "Autopulse" (Fig. 1), a device used to pump fuel from the tank to the carburetor of internal combustion engines. It may be obtained at a reasonable price through dealers in electrical automobile equipment. The device consists of a small metal bellows pump of about 0.8 c.c. capacity intake and outlet valves. The bellows is filled by the action of an electromagnet, which pulls against a spring. The spring expels the contents of the bellows at a constant pressure, and at the end of the stroke, contact is closed, which actuates the electromagnet once more. Current is supplied by a storage battery.

To adapt the "Autopulse" to physiologic purposes, it is necessary in the first place to introduce a means of adjusting the pressure. This is done by placing a thin nut between the spring and the arm which supports it and threading a machine screw into it. The end of the screw projects through a hole drilled in the bottom of the sheet metal casing. By turning the screw, the pressure may be adjusted from about 80 to about 250 mm. of mercury.

Next it is essential to arrange an electrical signal to register each stroke of the pump. This is done by soldering the tips of a separate pair of wires in shunt with the electromagnet. The wires run to a signal magnet on the special kymograph to be described below.

Finally, a constant supply of blood from a heparinized animal must be provided. This is derived in the present instance from cannulas in both fem-

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oral arteries. To insure that the amount necessary for each stroke is always available and that no more than essential is abstracted from the circulation, a float valve is required. The float chamber consists of a glass tube about 2.5 cm. in diameter, closed at one end by a two hole stopper. A short piece

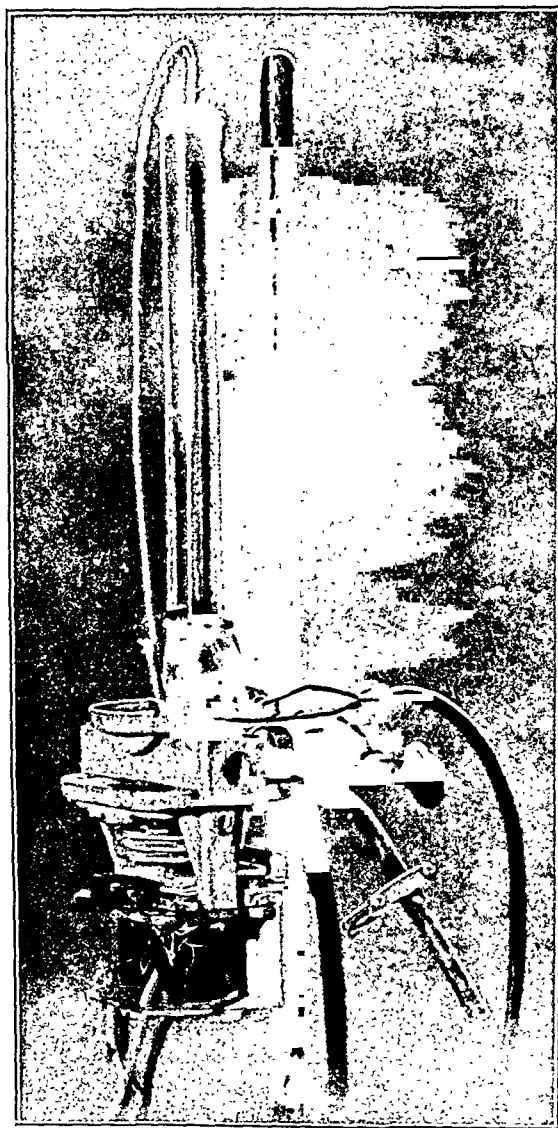


Fig. 1.—“Autopulse” provided with glass float chamber and float (above). To the right, leading to the float chamber, is the rubber tube and check valve. The outlet, connected to the carotid, is on the further side. The black wires lead to the signal magnet, the white wires to the battery. Notice that both are attached to the same terminal at the base of the solenoid, but that they are attached to opposite sides of the contact points above.

of copper tubing occupies one hole, and is soldered into the intake valve chamber of the pump (after the gasoline filter has been removed, and its projecting support sawed off level). An L-tube leads into the other hole, carrying at its further extremity an improvised cut-off valve. As is illustrated in Fig. 2, this

consists of a length of thinnest rubber tubing, 3 mm. in diameter, which runs between two wire rings fixed permanently in line. On a straight stub of wire at right angles to the axis of the tube, a wire crank is pivoted in such a way that its short arm compresses the rubber tubing when its long arm is elevated. The long arm is actuated by a J-shaped wire, which hooks over the side of the float chamber and rests upon the float. The float consists of a paraffined wood or pasteboard box (a small shaving-soap box would do) 5 to 10 cm. long, fitting easily in the float chamber. When it rises on the column of blood in the chamber, the flow from the femoral is cut off.

It is difficult to guard against the entrance of bubbles of air into the pump. To prevent embolism, the blood is passed from the outlet of the pump into a small inverted bottle, of about 20 c.c. capacity, and out again to the carotid cannula. If any air collects at the top of the bottle, it may be removed by a lumbar puncture needle inserted through the cork. A thermometer also lies in this chamber.

In some experiments the blood was warmed by passing through a coil of fine brass tubing in a thermostated jar; but a much simpler means of heating the air trap, such as an electric bulb placed near it, will suffice for most purposes.

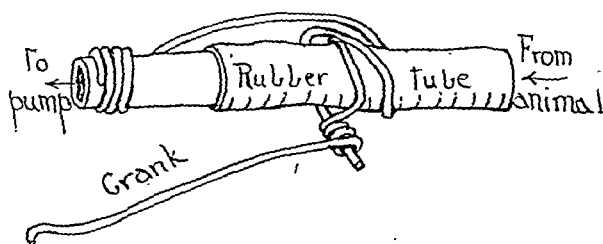


Fig. 2.—Diagram of the shut-off valve attached to the float.

The systolic and diastolic pressures at which the blood is delivered are read off on a mercury manometer, connected to the outlet by a T-tube. The carotid pressure and temperature are not registered directly, as they should be constant, but are written into the record from time to time during the experiment.

The output of the pump varies slightly with different pressures and should be measured directly under the particular conditions which exist for each series of experiments.

Before this perfusion pump had been a week in use, it became obvious that some special means of recording would have to be developed. The paper on an ordinary long kymograph was used up before a base line was established, unless it was run so slowly that individual strokes of the pump were difficult to distinguish, and the ordinary ribbon recorders are not adapted to register alterations in blood pressure. The following apparatus was therefore devised (Fig. 3) using inexpensive ready-made materials whenever possible.

The whole kymograph is mounted on a board 10 inches wide and 30 inches long. Rolls of adding machine paper, three and a half inches wide,

are used to take the record. The paper is suspended on a roller at one end of the board and passes at once under a bridge which carries two of the signal-magnets. It then runs far enough to allow the inked record to dry, before reaching the propelling mechanism.

The kymograph mechanism itself is built mostly from the gear box of an "Erector" set, obtainable at any toy shop. The paper is pulled by three wheels each composed of two of the discs intended to form the wheels of the toy automobile which can be built from the set. Slipping is prevented by flat "tires" of appropriate-sized rubber bands cemented to the circumference. The strip of paper slides upon a piece of celluloid which is bent around the front of the wheels so that the paper is in elastic contact with a full quadrant of their surface.

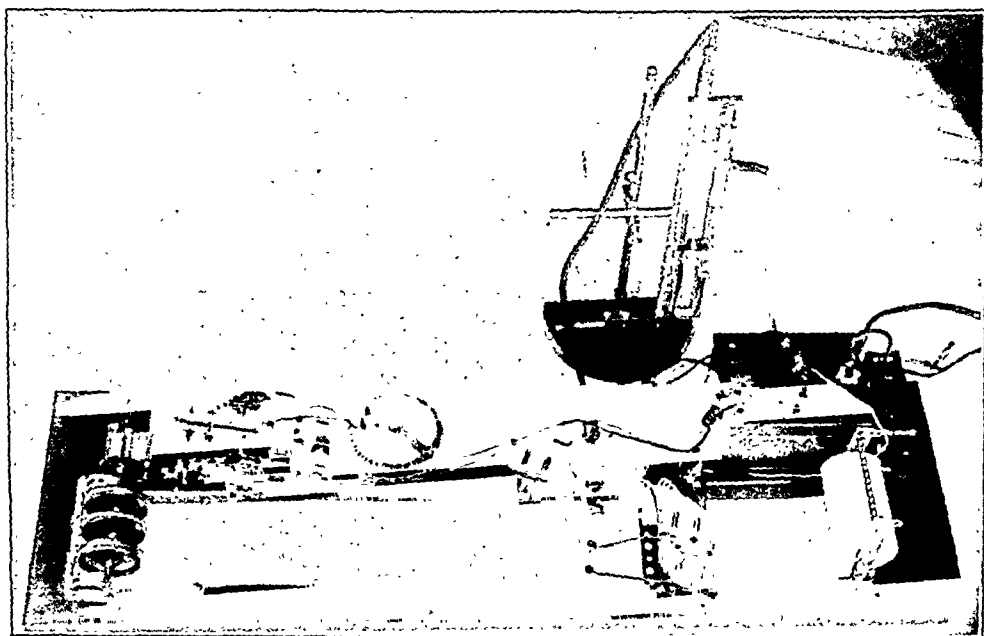


Fig. 3.—The kymograph assembled. At the left are the propelling wheels. Notice that the paper is curved around them. Above are the gear box and motor with rheostat. The connection between the mercury manometer and syringe used as recorder is shown. At the right is the transformer and electric clock.

The wheels are fixed between bearings to a length of shaft which extends into the gear box of the "Erector" set. The gear box affords a quadruple reduction of speed including a worm gear, but this is not enough for the purpose. In the present set the motor of an automobile windshield wiper has been used which contains an additional triple reduction within itself, and is operated by a transformer. A small motor with a worm gear reduction incorporated in it, such as may be obtained from laboratory supply dealers, would be preferable. Some sort of rheostat speed control is essential.

The transformer in the present set is also used to operate the signal magnets. There are three of these: one in shunt with the "Autopulse" pump, one to mark time, and one to record the application of stimuli, administra-

tion of drugs and the like. They are made from ordinary electric bells (of the best grade) with the bell, knob, cover and stamped base removed. For writing points, the smallest sized Keuffel and Esser lettering pen tips are used. The shank is bent into a sleeve to fit the vibrator of the bell. These writing points should be washed after use and kept in alcohol to prevent corrosion. If it were desired to eliminate the transformer, the signals could be operated from the storage battery with appropriate resistance interposed.

The timer is made of an electric clock with a sweep second hand. The best method of converting it to scientific purposes varies with the model of clock, but platinum contact points should be used in any case. In the present device an interval of fifteen seconds is convenient.

Blood pressure is recorded continuously from a mercury manometer connected by a T-tube to the intake of the pump and the femoral arteries. A thick-walled rubber tube passes from the distal end of the mercury manome-

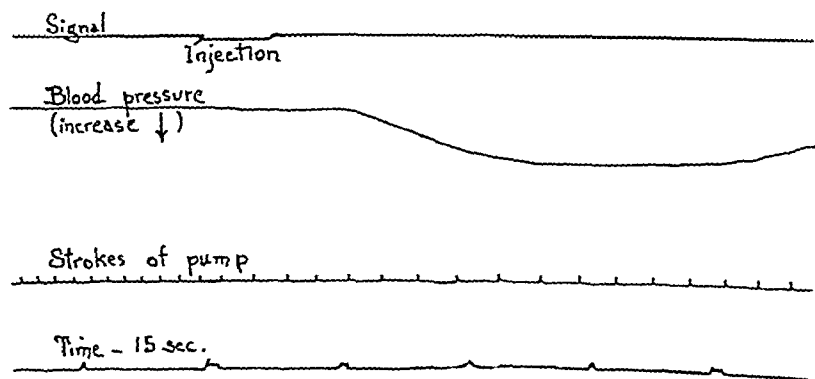


Fig. 4.—Sample of record. Perfusion of cat's common carotid under amytal. Effect of injection of 1/10,000 adrenalin into carotid: increase of blood pressure, slowing of strokes of pump.

ter to the tip of a 5 c.c. syringe. The space between the top of the mercury column and the piston of the syringe is filled with 50 per cent glycerine, and the plunger of the syringe is oiled. Any movement of the column of mercury is transmitted to the piston of the syringe but the resulting excursion is inversely proportional to the squares of the radii of the two tubes respectively. The syringe is fastened horizontally at the edge of the strip of paper and a writing point secured to the piston. It is possible to obtain tracings of individual pulse beats by this arrangement, although of course on a reduced scale.

The advantages of this kymograph are that it is inexpensive, portable, and compact. The timing device is self-contained. Records may be taken continuously over several hours and will show several signals simultaneously as well as recording blood pressure. Annotations may be made directly on the paper at the time they occur obviating the inconvenience of a separate set of notes.

A PIPETTE WASHING DEVICE*

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THE washing of the numerous pipettes required in the routine and research work of biologic laboratories is a vexatious and time-consuming detail. For a number of years we have employed for this purpose, and with much satisfaction, the device illustrated in Fig. 1.

The essential feature of the apparatus is the brass nipples threaded into lines of $\frac{3}{8}$ -inch or $\frac{1}{2}$ -inch brass pipe. Brass rod of $\frac{1}{4}$ -inch diameter is cut

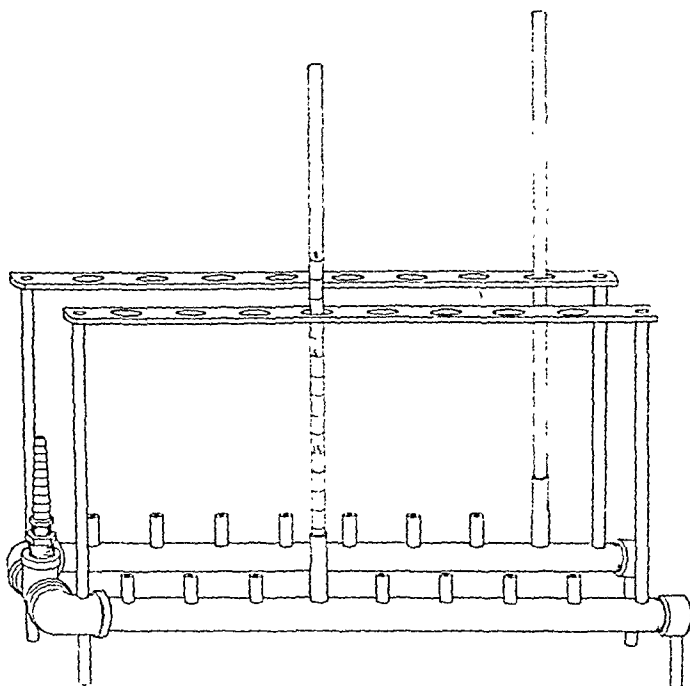


Fig. 1.

into $\frac{3}{4}$ -inch lengths. These are threaded at one end and drilled from the opposite end with a $\frac{1}{8}$ -inch drill, the final 2 or 3 mm. at the threaded end being drilled through with a $\frac{1}{32}$ -inch drill. When these nipples are screwed into water lines the small diameter of the bottom openings suffices to maintain sufficient pressure in the system to force water through the tallest pipette even though only one pipette may be in the apparatus. The nipples are fitted with short lengths of rubber tubing which receive the tips of the inserted pipettes. In Fig. 1 only two of the nipples are shown with rubber connections

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and holding pipettes. Some suitable support, such as that illustrated, must be provided for the upper portion of the pipette. Only a moment's time is required to insert a pipette, and washing with constantly changing water then continues automatically until the pipette is removed.

The apparatus pictured is designed to be connected by rubber hose to a water cock, and may be moved from one sink to another. In our laboratories we have found it convenient to have a single line of nipples permanently installed near the bottom and at one end of each sink where the device interferes least with other uses of the sink.

A surprisingly large number of nipples may be assembled in a single unit without loss of efficiency. Since, however, water escapes from every outlet whether or not these are occupied by pipettes, a larger capacity unit than normally sufficient leads to an unnecessary wastage of water.

Test tubes, centrifuge tubes, and other similar pieces are conveniently and automatically washed in this apparatus by inverting them over glass tubes connected to the nipples.

THE CALIBRATION OF WHITE BLOOD CELL DILUTION PIPETTES*

F. LOWELL DUNN, S.B., M.A., M.D., OMAHA, NEB.

THE technical skill required in the manufacture of blood cell dilution pipettes must excite the admiration of all who give thought to the problem. Obviously such micro pipettes must show variations in precision. This fact is recognized by the manufacturers, some of whom provide factory certificates and guarantees of accuracy or individual certification either by themselves or by the Bureau of Standards. Textbooks of clinical pathology show a widely varied response to the possibility of pipette errors. At the one extreme is the statement that only pipettes certified by the Bureau of Standards should be used in clinical laboratories, while at the other extreme is the complete omission of any comment regarding the possibility of such errors. Probably the majority of physicians assume dilution pipettes as purchased to be sufficiently accurate for clinical purposes, or check them against other pipettes to maintain uniformity. Using the same pipette for consecutive blood counts on the same patient is a common device for ascertaining relative changes in leucocyte counts. This is a tacit admission of error in apparatus and is at best an inconvenient method in laboratories where a large number of pipettes are in daily use.

The extra charge made for reliable certification seems most reasonable to anyone who has undertaken such calibration. There is, however, a general impression among clinicians and hospital administrators that such certification is warranted only for purposes of academic research.

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The Bureau of Standards specifications for dilution pipettes with a 1 to 10 ratio require (in addition to certain details of construction) that the dilution must not be in error by more than ± 3.5 per cent. This is, of course, the pipette error alone and does not include other possible sources of error such as, (1) technical manipulation of pipette and hemocytometer, (2) physiologic variations, and (3) errors in the construction of the hemocytometer. Since rises in leucocyte counts of 1000 to 2000 per cubic millimeter are often determining factors in formulating opinions regarding treatment, a method error of 10 per cent looms large and an error of ± 3.5 per cent in the pipette alone is a large fraction of this total error.

The accidental discovery of a defective pipette in our own service raised the question of the accuracy of dilution pipettes in current clinical use. New lots of pipettes were supposed to be checked against older lots for consistency but this had been neglected because of lack of time. We were unable to obtain any impartial information and the discrepancy in textbooks of clinical pathology made it desirable to determine whether the extra cost of calibration was a justifiable expenditure. Through the courtesy of several physicians we obtained for calibration 86 white cell dilution pipettes.

METHODS

After inspection the pipettes were rinsed with nitric acid, bichromate cleaning mixture, water, alcohol, and ether until examination showed them to be clean.

Gravimetric.—The weights of distilled water or 1 per cent fuchsin included between the tip and the "0.5" mark, and between the tip and the "11" mark were determined. The necessary precautions in calibration of volumetric ware were observed.

This method was slow and since the weight of water included between the tip and the "0.5" mark was often not over 10 mg. it was necessary that the manipulations required in filling the pipette and the weighing itself must not introduce errors of over 0.1 mg. to determine the pipette error within 1 per cent. The necessity for technical skill and experience was shown by the amount of practice required before consistent results could be obtained, probably making this method available only to those who had survived the equivalent of an elementary course in quantitative analysis.

Peffer Method.—A much more promising method was that of E. L. Peffer of the Bureau of Standards.¹ His excellent instrument eliminates the handling of the pipettes during the calibration, requires considerably less skill, and is five to ten times faster than the gravimetric method. To construct a duplicate of his fine instrument was prohibitive, but it was obvious from his description that the basic features could be satisfied at a negligible cost.

The device, as assembled by us, is illustrated in Fig. 1 with the protective cotton cover removed. It consists of a small reservoir containing 1 per cent fuchsin, a one hole rubber stopper to fit the bulb end of the pipette, and a piston by which the fuchsin can be forced up into the pipette, the volume of liquid being measured by a micrometer screw feed on the piston. The pipette is placed vertically in the stopper, with the tip up. The solution is forced to the "11" mark and a reading is taken. It is then forced up to the "0.5" mark and to the tip, readings being made at each level. The result is a measurement of the desired volumes of the pipette in terms of micrometer screw piston displacements from which the dilution value of the pipette can be quickly calculated. The small reservoir in our apparatus was a Pyrex tee. A Brown and Sharpe micrometer head was cemented into one end of the tee with De Khotinsky cement. The other end was closed with cement. The tee was used in order to secure rigid clamping to the ring stand by the usual laboratory supports. The upright portion of the tee was fitted with a rubber stopper. A single hole was bored,

the size being selected to fit tightly the bulb ends of the pipettes to be calibrated. The inner end of the rubber stopper was made concave so that air bubbles would not be trapped, but could be forced out through the hole in the stopper. The system was then filled with 1 per cent fuchsin and all air bubbles carefully expelled.

Slight fluctuations of temperature of the fuchsin solution produce marked variations in the height of the solution in the capillary tube of the pipette. Consequently the apparatus must be carefully shielded. Peffer shielded his apparatus by a vacuum container. We wrapped the tee with several layers of cloth before clamping it to the ring stand, and then surrounded tee, clamps, and part of the ring stand with cotton, leaving only the rubber cork and the micrometer head exposed. The cotton was put on in layers to a thickness of two to four inches, being careful that all air channels were closed. The knurled portion of the micrometer head was covered with several wrappings of cloth to protect it from the heat of the hand. This protection was sufficient if drafts were avoided, although on a few days

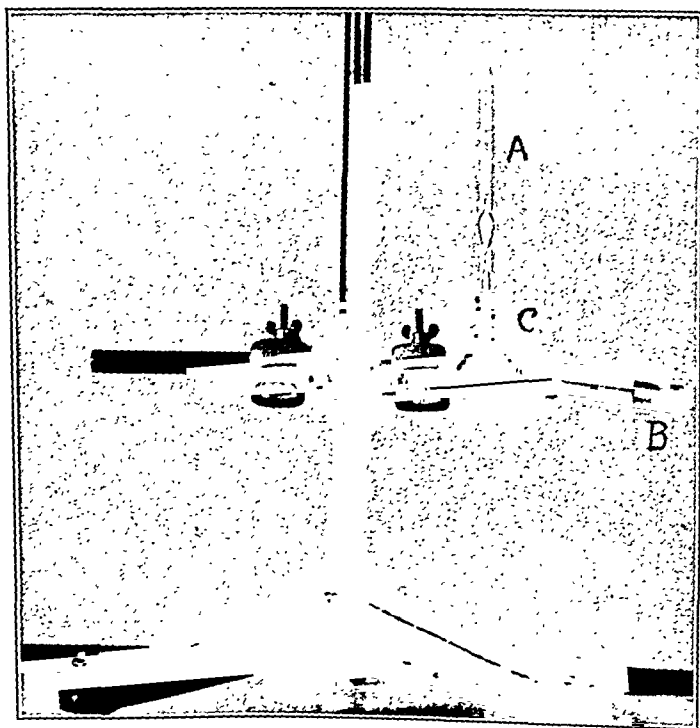


Fig. 1.—Dilution pipette calibrator for white blood cell pipettes utilizing some of the features of Peffer's instrument. A, white pipette; B, micrometer head; C, tee reservoir. The cotton cover to minimize thermal effects has been removed.

temperature fluctuations made consistent readings impossible. Later, by partly filling the tee with hard paraffin, the volume of liquid was reduced from about 30 c.c. to 8 c.c. and the temperature effect was definitely less marked.

Several practical details developed during use. The rubber stopper should fit the tee and the pipette tightly and without any tendency to crawl. It was preferable to cement the stopper to the tee. Any crawl was apparent in duplicate readings, or the shift in the fluid level could be observed after waiting a minute or two. The crawl could be accelerated by gently tapping the pipette with a pencil. After setting the level at the "11" mark, it proved useful to tap the pipette to minimize any crawl during the subsequent readings. Bubbles occasionally adhered to the glass bead in the bulb of the pipette. These should be watched for very carefully during the filling of the bulb and should be removed by tapping.

Any air in the system caused erratic results and lag in response when the piston was moved. This was due to incomplete filling of the reservoir or to failure in keeping the

hole in the stopper sufficiently filled with fuchsin. The reservoir was kept filled by dropping the fuchsin solution on the stopper and drawing it in by means of the micrometer screw. The pipettes must of course be dry for each set of measurements, although measurements of the interval between the tip and the "0.5" mark could be duplicated usually without drying between determinations.

The meniscus should be set flat across the orifice when the setting at the tip is made. Many pipettes show a constriction in bore at the tip, and it is our experience that lag due to small amounts of air show up most conspicuously in this region. The setting at the tip is best made with the aid of a magnifying glass.

The method described above proved much more satisfactory than the gravimetric. If the reservoir is so designed that air bubbles are not trapped, the system is easily filled. Skill in attaching the pipettes is quickly acquired, and it is easy to control accurately the level of the solution by the micrometer screw. This method measures the pipette error directly and does not include the errors of filling and handling the pipette as in the gravimetric method.

Colorimetric Method.—The dilution value of the pipette can be measured directly in a colorimeter against a standard dilution. Several colored solutions were tried and 20 to 30 per cent chromic sulphate was selected because of the high sensitivity of the eye for green and because twenty-two fold dilution still gave a satisfactory intensity in the colorimeter at a cell depth of 20 mm. The standard dilution was a 1:22 dilution of the strong solution. The concentrated chromic sulphate solution was sucked up to the "0.5" mark and then diluted to the "11" mark with distilled water. After shaking a moment the entire contents were discharged into a colorimeter cup. This process was repeated, drying the pipette each filling until sufficient solution was obtained to give an effective depth of 20 mm. With microcolorimeter cups one to three fillings were sufficient, the larger all-glass cups requiring four or more fillings. This might appear to be a drawback in the method, but it is compensated by the fact that the colorimeter reading is an average of several fillings.

The evaporation of the liquid in a glass microcolorimeter cup was measured and found to be about one milligram in fifteen minutes at 27° C. on a dry day when the cup was covered with a watch glass. Since the total volume of leucocyte pipettes is usually 0.25 c.c. or more, this loss is not significant. Furthermore with a suitable suction pump and the use of alcohol and ether the pipettes can be cleaned and dried in a minute or two so that three fillings and the necessary cleanings can be accomplished within ten minutes. A 30 per cent chromic sulphate solution was tested for change of color over the temperature range of 24 to 45° C. None was observed.

The green color can be very satisfactorily matched in the colorimeter, but faulty use of such instruments is so common that several details should be mentioned. The zero settings should be carefully checked. The colorimeter should be symmetrically placed with respect to the illumination. Any inequality in the fields should be located and rectified and not compensated by twisting the position of the instrument with respect to the light. If the plungers are not dry, they can be rinsed with the standard solution since it closely approximates the strength of the unknown. When a series of pipettes are to be calibrated, the standard solution should be changed frequently enough to avoid appreciable evaporation. The colorimeter light should be turned off when readings are not being made.

This colorimetric method appeared to us logical and was much easier to do than the difficult gravimetric method. Colorimetric methods are more familiar to the clinical laboratory worker than gravimetric. The hydrometric method of Peffer is perhaps not so applicable to general use because of this drawback of unfamiliarity. The colorimeter method showed no greater variation in our hands than those obtained with the use of our makeshift micrometer screw calibrator. With the increase in ruggedness and precision of photoelectric circuits the adaptation of the colorimetric method to a photoelectric one is an obvious technical procedure. Since the colorimetric readings for certifiable pipettes are within ± 3.5 per cent, it might

prove unnecessary to use a photometric head, the readings being made by galvanometer deflection. The speed of such a device should materially reduce the cost of pipette calibration.

The calibration of the various pipettes was controlled by: (1) repeated checks on different days, (2) check against the gravimetric method, and (3) comparison with the Bureau of Standards certified pipettes. Three or more readings were averaged. Most of the readings were made with the micrometer screw calibrator.

DISCUSSION

A few of the pipettes were discarded because of tips broken sufficiently to affect the accuracy, leaving a total of 84 to be discussed. Of these 10 bore no visible identification mark while the rest included 13 different distributors' names or identification marks. All but 18 had a serial number. We do not know how many manufacturers were represented in this series of 84 pipettes, but we are informed that the manufacture of these pipettes is a highly specialized industry and confined to very few firms. Only one pipette

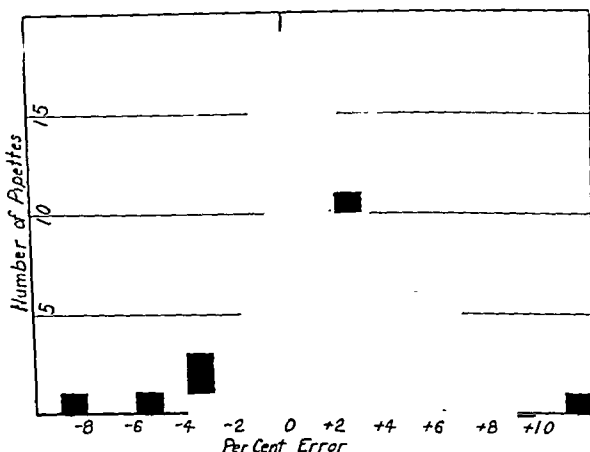


Fig. 2.—Frequency curve of errors of 84 white blood cell dilution pipettes.

had been recognized as being in error prior to our calibrations. One pipette, not included in the series, had no mark whatever on the capillary stem. We were unable to associate defective pipettes with any particular identification mark. It was possible to obtain catalogue specifications, guarantee, or factory certificates on 63 pipettes. The small size of our series does not permit in any sense a general statement but with this qualification the nature of the guarantee or specification had little or no bearing on the accuracy of the pipette except in those cases where there was an explicit statement that the pipette had been individually calibrated. All pipettes that had been individually calibrated were within the tolerance stated. Under present conditions the inference can be tentatively made that individual calibration is necessary to eliminate defective pipettes.

The results of our calibrations were plotted in a frequency curve to conserve space (Fig. 2). Of the 84 tested, 24 (28 per cent) showed errors in excess of ± 3.5 per cent. Of these, 10 (11.5 per cent) were in the group 3.5 to 5 per cent, 12 (14 per cent) in the group of 5 to 10 per cent, and 2 were over

10 per cent, one of which had an error of 38 per cent. It is interesting that plus errors were more common than minus errors and in the ratio of 68 to 16. Since a portion of the calibrations were done both by the micrometer screw piston method and by the colorimetric method we were unable to explain the frequency of the positive errors on the basis of the method of calibration.

With 28 per cent of the pipettes showing errors in excess of ± 3.5 per cent, the possibility of serious errors arising in laboratories where a large number of pipettes are in use is far from negligible even for clinical purposes.

SUMMARY

1. Calibration of 84 white blood cell dilution pipettes in clinical use showed 28 per cent to have errors in excess of ± 3.5 per cent, 11.5 per cent to have errors of 3.5 to 5 per cent, and 14 per cent to have errors of 5 to 10 per cent with two having errors of 11 per cent and 38 per cent respectively.

2. Positive errors were more common than negative in the ratio of 68 to 16.

3. A colorimetric method for the calibration of dilution pipettes has been described.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PERTUSSIS, Production of Antipertussis Serum of High Titer, Bailey, J. H. *J. Infect. Dis.* 52: 97, 1933.

By repeated intraperitoneal injections into cocks of massive doses of a suspension of live, virulent pertussis bacilli, an antipertussis serum of high titer, not causing serum sickness even in large doses, was produced, which, in the limited trial afforded it, gave evidence of being of value in the treatment of pertussis in children. No definite statement as to the therapeutic value of this serum may be made until a greater number of patients have been treated.

SYPHILIS, Serodiagnosis of, Denison, G. A., and McDonald, E. G. *Am. J. Syph.* 17: 90, 1933.

Serologic tests for syphilis because of their complicated and empiric nature are subject to variation, and the use of standardized technics in different laboratories does not mean that comparable results will be obtained. Each clinician should have a very definite idea of the clinical level of the tests performed for him. Clinician and serologist should work together, each sympathetic of the other's problems.

Various tables are compiled showing comparative results obtained with several methods as used in a board of health laboratory. However, it should be emphasized that similar results would not necessarily follow in other laboratories using the same methods. For this reason only very generalized deductions should be made.

A close comparison of methods cannot be made by tabulating results on all specimens from cases of a general nature since the large number of negative routine examinations minimize the differences. True comparisons are best made on treated and untreated syphilitic cases. Finally, where results disagree a close check of clinical findings should be made to determine the correlation. Judged in this way the apparent working agreement of 93.6 per cent obtained on all specimens with four antigenic tests is reduced to 89.1 per cent in untreated and 79.5 per cent in treated syphilis. The greatest number of disagreements occur in recently treated cases. However, there are always a small number of syphilitic cases which for some reason are positive only with one method or another.

The authors' experience justifies the conclusion that no single test is ideal for confirmatory diagnosis and treatment of syphilis, that none of the technics studied have any inherent specific qualities, and that the best results may be expected from the use of multiple tests which include at least one precipitation and one complement fixation method. The authors believe that multiple tests allow greater latitude in the quantitative estimate of the patient's serology, and, thereby, serve as a better guide for treatment.

PNEUMONIA, Specific Serum Treatment of Type I, Parsons, J. W., and Sutcliff, W. D. *Am. J. M. Sc.* 186: 52, 1933.

The effectiveness of two methods of dosage of specific serum in the therapy of pneumococcal Type I lobar pneumonia is compared in two groups of 12 cases each, treated before the fifth day of their disease. In the first group amounts of serum were given (15 to 105 c.c., average 45 c.c.), just sufficient to maintain agglutinins in the circulating blood, as determined by a rapid bedside method. In the second group a larger, empirically selected dose (75 c.c. or more, average 79 c.c.) was given to each patient.

In both groups the response to the therapy was satisfactory as shown by the short duration of the disease after therapy, and by the death rate. On the average, the course of the disease in the 12 empirically treated patients was much shorter than the average course of a larger series of cases treated in the same manner, but this unusual result in a small series of cases does not necessarily indicate the superiority of this method of therapy.

Evidence is given to indicate that the persistence of agglutinins for the hemologous organism is an index that a sufficient amount of antipneumococcic serum has been administered to produce a satisfactory therapeutic result.

TUBERCULOSIS: The Question of Tubercle Bacilli in the Blood in Advanced Pulmonary Tuberculosis, Corper, H. J., and Damerow, A. P. *Ann. Rev. Tub.* 28: 118, 1933.

Tubercle bacilli were not found to be present in the blood of 120 patients with advanced pulmonary and generalized tuberculosis at the National Jewish Hospital and Fitzsimons General Hospital at Denver, Colorado, in spite of the fact that each blood specimen was submitted to four different methods of examination for tubercle bacilli, namely, guinea pig inoculation, and three different culture methods. While control tests proved the efficacy of the culture methods used for discerning small numbers of tubercle bacilli in human and animal blood, acetic acid, as recommended by Loewenstein, destroyed small numbers of human tubercle bacilli, while saprophytic acid-fast bacilli would survive such treatment, thus vitiating the results obtained with this reagent.

In addition to the 120 cases studied, over 200 routine smaller specimens of blood obtained from patients in a tuberculosis sanatorium and examined by means of two reliable culture methods, the tissue-substrate method and the sulphuric acid crystal violet potato cylinder method, did not reveal a single positive culture for tubercle bacilli among these specimens. As a result of the examination of the blood from the 120 cases of advanced tuberculosis two specimens yielded saprophytic acid-fast bacilli, which could readily be differentiated from pathogenic tubercle bacilli by animal inoculation and reliable culture methods.

Tubercle bacilli were found to grow well when present in small numbers on a sulphuric acid treated and sodium bicarbonate or sodium hydroxide neutralized blood residue or on inspissated defibrinated blood, making it appear that the acid- or heat-treated or sterilized blood pigments are not detrimental to the growth of tubercle bacilli. The macroscopic type of culture obtained on the blood medium differs from that seen on potato or on egg, which also differ from each other.

The Congo red potato flour egg medium (Loewenstein) is a good one for human and bovine tubercle bacilli, but it is no better than a simple potato cylinder or inspissated egg yolk medium, and it possesses the disadvantage of being unnecessarily encumbered with expensive and unessential ingredients.

It appears that human or bovine tuberculo bacteremias, in the sense that tubercle bacilli circulate in the blood for a fairly long time or that the bacilli multiply in the blood, are not borne out by these studies, although it is not intended to convey the impression that occasional embolic disseminations (showers) from disintegrating tuberculous foci do not occur or that there may not exist terminal periods in the course of the disease when numerous showers of caseous products containing viable bacilli may make it possible to find them in the blood of man and experimental animals. However, such a condition of tubercle bacilli in the blood is far from being the common event, according to the observations made in this study.

It would appear also that embolic showers (embolemia), when they do occur in man, are rapidly removed from the circulation in the usual case of tuberculosis.

DIPHTHERIA: Active Immunization Against, by a Single Injection, Strauss, H. W. *J. A. M. A.* 101: 192, 1933.

A concentrated diphtheria toxoid incorporated in hydrous wool fat has been injected intramuscularly and actively immunized 99 per cent of 103 patients within a period of two

months. In the majority of cases, immunity developed in from three to four weeks and, in one case, as early as two weeks.

Because of the early development of active immunity, a preparation such as that described may have a practical usefulness in checking an epidemic of the disease. A single injection method has obvious practical advantages.

The material used in this study was prepared in the following manner: Diphtheria toxoid (M.L.D. 500 per c.c.; L. 0.13; L.F. 0.1), 7,300 c.c. was placed in cellophane bags and fanned at room temperature until the volume was reduced to 250 c.c. The dark colored granular precipitate that formed was removed by centrifugation. The supernatant fluid was diluted up to 730 c.c. in the process of twenty-four-hour dialysis in a cellophane sack and by the addition of distilled water. Phenol (0.5 per cent) was added and the solution was filtered through a Berkefeld candle. The least fatal dose of the concentrated solution was 0.014.

In a similar way, the solution was further concentrated to 73 c.c. (100:1 concentration). This was preserved, after Berkefeld filtration, with merthiolate in a final concentration of 1:10,000. This concentrated fluid was found to contain 300 flocculating units per cubic centimeter.

Two batches of the lanolin mixture were prepared as follows: Batch 301-H-11 was made by thoroughly mixing, at 42 C., 100 c.c. of sterile hydrous wool fat and 50 c.c. of concentrated diphtheria toxoid. One cubic centimeter of this mixture thus contained 100 flocculating units and 0.2 c.c. contained 20 flocculating units. Batch 301-H-12 was made in the same way as 301-H-11 with the exception that, to each quantity of 0.2 c.c. of the mixture, 0.05 c.c. of sterile olive oil was added.

The material was received in individual miniature antitoxin syringes, each containing a single dose. The preparation is of the consistency of a soft wax and is easily expelled from the syringe.

No reactions were observed in children under five years of age. In the school age group there was an occasional complaint of soreness in the arm for a day or two with slight local redness and in one case a temperature of 101 during the first twenty-four hours. In the adult group, five who had positive Schick but negative control reactions were included in the series. They complained only of a heavy feeling in the arm. Five other adults (not included in the series) who gave positive Schick and control tests were given the injection. Of these, two gave severe reactions with a rise of temperature to 102, headache, malaise, a large area of local redness with induration, and regional adenitis. The fever subsided in twenty-four hours. The three remaining persons had mild local reactions without constitutional symptoms. The reactions to be expected, then, are the same as those obtained with ordinary toxoid. They are infrequent and quite mild in older children and absent in those of preschool age. The more severe reactions are to be expected in those patients who give positive reactions to both Schick and heated control material.

PERTUSSIS: Occurrence of the Bordet-Gengou Bacillus, Kristensen, B. J. A. M. A. 101: 204, 1933.

The results of this study are conformable with the hypothesis that the Bordet-Gengou bacillus is the etiologic agent of whooping cough.

The cough-plate method has been used for sixteen years at the State Serum Institute in Copenhagen and has proved to be of practical value. It is the best method for diagnosing the disease early.

An isolation period of four weeks after the onset of the typical paroxysm has in practice been sufficient for school children.

Healthy carriers have not been found outside of families in which whooping cough exists.

Abortive and quite atypical cases of whooping cough are frequently found, and the author believes they play an important rôle in the spread of the disease.

PUERPERAL INFECTION: Relationship Between Exogenous Throat Streptococci and Kellogg, F. S., and Hertig, A. T. *Trans. Am. Assn. Obst. Gynec. & Abd. Surg.* 87: 1932.

From a study of an epidemic in the maternity wing of a well-equipped general hospital in 1931, it is concluded:

That nasopharyngeal carriers of hemolytic streptococci (and perhaps other organisms) are a most dangerous source of frequently fatal sepsis to women in childbirth from the moment labor begins (and possibly before), to the end of the puerperium (at present an unassignable date but at least fourteen days after delivery).

That "silent carriers" are potentially as dangerous as persons acutely sick with temperatures, save for less likelihood of coughing or sneezing, although, usually, a history of some acute exacerbation in the not distant past may be obtained from the carrier.

That perineal contacts are presumably the most frequent method of transmission; either by coughing, sneezing or talking with the patient during perineal precautions or by incomplete manual asepsis after self-infection of the hands by the carrier, as by the use of a handkerchief; but that for the present other less direct methods must be assumed, and guarded against.

Hence, that every carrier is a source of danger and must be eliminated or be masked adequately at all stages of labor and the puerperium and be absolutely trained and trustworthy about digital asepsis.

That since pupil nurses are untrained and unhabited to conscientious digital asepsis, it is a wise precaution to culture each one on entrance to a maternity hospital and to eliminate each one temporarily until the throat is negative for hemolytic streptococci.

That other personnel, i.e., doctors and graduate nurses trained in asepsis, following rules akin to those given above, will in all probability not prove a hazard to patients.

That the question of ward attendants, especially those serving food, must be carefully considered.

That a single case of puerperal sepsis in an institution calls for immediate rigid investigation and drastic measures based on this to limit spread.

MILK: The Use of Glycerol as a Preservative for Specimens to Be Examined for Hemolytic Streptococci, Gilbert, R., and Clark, M. E. *Am. J. Pub. Health* 23: 720, 1933.

The addition of glycerol to make a 30 per cent concentration has been found to enable recovery of streptococci from milk after seven days at room temperature or after ten days' refrigeration. It is thus of value in the study of epidemics of septic sore throat.

THYROID DISEASE: Effect of Treatment on Blood Cholesterol in, Hurxthal, L. M. *Arch. Int. Med.* 521: 86, 1933.

The blood cholesterol is low in toxic thyroid states, and is brought to a normal level partly by preoperative treatment but chiefly by subtotal thyroidectomy.

The reciprocal relationship between the average elevation of the basal metabolic rate and the average lowering of the blood cholesterol level in toxic goiter, is further confirmed by the results of treatment presented in this paper.

There appears to be little difference between the change in the blood cholesterol values in exophthalmic goiter and that in toxic adenomatous goiter as the result of treatment.

The blood cholesterol in nontoxic adenoma or adenomatous goiter undergoes little or no change as the result of removal of the growth.

The blood cholesterol determination is of distinct but limited value in the diagnosis of hyperthyroidism.

BLOOD CULTURES: Comparative Study of, Taken With Kendall and Routine Mediums. Friedberg, C. K. Arch. Int. Med. 52: 120, 1933.

One hundred and three routine blood cultures were made for 77 patients suffering from a variety of febrile illnesses suggesting the presence of bacteremia. Simultaneously, flasks containing K medium were inoculated with blood from these patients.

The K medium showed 4 per cent less positive results than the ordinary routine method. Compared with the individual flasks and plates used in the ordinary method, the flask of K medium showed slight inferiority to some and slight superiority to other individual units.

Other disadvantages were found in the difficulty and expense of preparing the medium and the greater time necessary before an organism could be identified.

Repeated subcultures from the original K flask failed to show any invisible, filterable forms which could be converted to the ordinary state by transfer to solid medium as alleged by Kendall. There was no evidence in the cases studied to show that the clinical picture was due to a filtrable, invisible form of an organism which did not show itself on the ordinary mediums.

In several instances common organisms were found to pass through the Berkefeld filter, suggesting the possibility that the K medium may have had some influence in impairing the efficiency of the filter.

LIVER FUNCTION: An Appraisal of the Modified Dextrose Tolerance Tests, Ricketts, H. J. Arch. Int. Med. 52: 147, 1933.

The test in question follows:

1. Estimation of blood sugar during fasting was made in the morning.
2. The patient was then given 20 units of insulin.
3. Twenty minutes later, the patient was given 50 gm. of dextrose in 500 c.c. of water by mouth, followed by 1,000 c.c. of water by mouth.
4. Blood sugar determinations were made at one-half hour, one hour, two hours and three hours after the ingestion of the dextrose and water.

The modified dextrose tolerance test of the function of the liver, as described by Althausen, Gunther, Lagen and Kerr, was performed on ten patients who were considered to have normal livers at the time of examination, and on four patients with frank hepatic disease.

Of the ten normal patients, nine responded to the test by a drop in blood sugar to points considerably below the critical level for normal reactions, and showed symptoms and signs of hypoglycemia.

Of the four patients with frank hepatic disease, three responded by definite chemical and clinical hypoglycemia.

There was no essential difference between the behavior of the normal and the abnormal groups.

The modified dextrose tolerance test in its present form is not a satisfactory measure of the metabolic function of the liver.

PREGNANCY, Hormone Diagnosis of Viability of, Spielman, F., Goldberger, M. A., and Frank, R. T. J. A. M. A. 101: 266, 1933.

Thirty-three pregnancy cases in which missed abortion was suspected or expected were studied from the hormonal aspect in order to determine the relationship between the female sex hormone of the blood and the pituitary hormone of the urine on the one hand, and fetal life or death on the other.

The Frank-Goldberger method was used in the blood studies, and either the Aschheim-Zondek or the Friedman test in the urine studies.

A negative female sex hormone reaction was obtained when the fetus was dead (23 cases) and a positive reaction when the fetus was alive (8 cases). The results here were 100 per cent correct.

A negative pregnancy reaction was obtained when the fetus was dead in eleven cases, and ten cases gave positive reactions in spite of dead fetuses (approximately 50 per cent correct).

The female sex hormone blood determination has proved itself in this small series an absolute indicator of whether a fetus is alive or dead. The urine pregnancy test is of value only when the reaction is negative.

The presence or absence of the hormones depends on the degree of involution of the placenta and its attachment to the uterine wall.

TUBERCULOSIS: The Tuberculin Patch Test, Grozin, M. Am. J. Dis. Child. 46: 17, 1933.

The tuberculin patch test is made by moistening a piece of adhesive plaster with 1 or 2 drops of tuberculin and applying it to the skin, which has previously been cleaned with ether. For control, a piece of adhesive plaster without tuberculin may be used. A positive reaction is indicated by redness studded with papules and vesicles.

A study of 176 cases showed that this method compared favorably with the Pirquet test as to reliability.

Its advantages over the Pirquet test and other tuberculin tests are:

- a. It is absolutely painless and therefore does not spoil the relation between child and physician, or frighten a neurotic patient or anxious mother.
- b. It requires no boring, puncturing, scratching or rubbing.
- c. It requires no sterilization of instruments, such as lancets, needles, and syringes.
- d. There is no danger of infection.
- e. It is of much simpler technic, so that it can be done by nurses without much training.

f. It is less time-consuming, since there is no waiting for the tuberculin to dry or to act on the skin, or watching that the child does not wipe it off or spread it to the area of control. It is therefore of advantage when large numbers are to be tested or in busy practice.

g. It is possible to control the area of reaction, since this is usually limited to the surface covered by the adhesive plaster. In other percutaneous tests, such as the Moro, the reaction cannot be controlled, once it has started, because the tuberculin must be rubbed into the skin, and friction of clothes tends to spread it still further. In the tuberculin patch test the reaction can be regulated by the size of the adhesive plaster, by the number of drops of tuberculin and by the strength of the dilution.

h. It is easier to interpret the results with the tuberculin patch test, because the area of reaction can be arbitrarily enlarged by applying larger pieces of adhesive plaster, more tuberculin and tuberculin of greater concentration.

PNEUMONIA IN INFANTS, Due to *B. Mucosus-Capsulatus*, Jerguson, J. A., and Tower, A. A. Am. J. Dis. Child. 46: 59, 1933.

Two cases of lobular pneumonia in infancy caused by *Bacillus mucosus-capsulatus* have been reported; the twins were seven months old, a girl and a boy. The girl contracted the disease first and died on the eighth day. The boy became ill two days after the onset of the illness in his sister but completely recovered after thirty-two days' hospitalization.

Bacteriologic examination of the throat and tracheal secretion is the most important procedure from a diagnostic standpoint. The extreme pallor of the skin and dehydration, the cough and mucopurulent nasal discharge in the two cases described in this report, together with the vomiting and diarrhea which cleared up early in the disease, are suggestive from a standpoint of differential diagnosis.

Pneumonia in infants due to *Bacillus mucosus-capsulatus* may not terminate fatally. The pulmonary involvement is, perhaps, most often lobular, and the roentgen signs which are characteristic of the disease in adults may not occur in infants.

HEMOGLOBIN, Content of Blood in Infants, Elvehjem, C. A., Peterson, W. H., and Mendenhall, D. R. *Am. J. Dis. Child.* 46: 105, 1933.

A hemoglobin curve for infants constructed from 2,000 determinations on 750 subjects varying in age from birth to five years is given.

The hemoglobin content of the blood of infants was found to be high (22 gm.) but it fell rapidly and reached a low point at the age of from eight to twelve weeks. There was an increase between the ages of twelve and twenty-four weeks, and a slight decrease thereafter. The hemoglobin remained between 11 and 12 gm. until the infants were about two and one-half years old, after which it increased gradually.

The rapid drop during the first few weeks of life and the low hemoglobin values recorded during the first six months were not prevented to any appreciable extent by the feeding of a good diet of natural foodstuffs. The hemoglobin values of the children on the special diet, however, began to increase at an earlier age than the average values for all the children.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Aetiology of Tuberculosis*

THIS is an English translation made by Dr. and Mrs. Max Pinner of Robert Koch's original communication on the etiology of tuberculosis which appeared in the *Berliner Klinische Wochenschrift* the tenth of April, 1882. The present volume was prepared by the National Tuberculosis Association to commemorate the fiftieth anniversary of the discovery of the cause of consumption. Dr. Allen Krause contributes an introductory appreciation of the epoch-making contribution of Koch. The booklet is illustrated with facsimiles of the first page of Koch's original paper and several other pertinent photographs.

Only two English translations have previously appeared, one in the *Canada Medical and Surgical Journal*, 1881, and the other in the *Cincinnati Lancet and Clinic*, 1883.

Koch's original contribution, long since a classic in medicine and an epoch-making paper, should long since have been available in this form and the translators and the Tuberculosis Association are to be congratulated.

Calcium Metabolism and Calcium Therapy†

AN EXHAUSTIVE and at the same time authoritative treatise on our knowledge of the metabolism of calcium and its use in the treatment of disease. While the major portion of the volume is given to those diseases in which there is a clear-cut error in calcium metabolism, the author also includes a discussion of quite a variety of diseases in which the use of calcium is certainly a moot question. The author of course recognizes this fact and outlines the treatment that has been recommended in this particular type of disease by those who claim to have obtained good results.

Outline of the Cranial Nerves‡

THIS is a small reference manual for students and for those of us who after a certain number of years find it difficult to recall the origin, distribution, functions, etc., of the cranial nerves. One of the author's innovations which appears to be especially logical is his process of tracing the sensory nerves from the sensory end organs backward centrally, rather than in the usual reverse. The motor organs he traces from the central point of origin of impulse to the periphery. One who has learned the cranial nerves with this method should be better able to keep them distinct in his mind.

*The Aetiology of Tuberculosis. By Dr. Robert Koch. A translation from the German of the original paper announcing the discovery of the Tubercle Bacillus, read before the Physiological Society in Berlin, March 24, 1882, and published in the *Berliner Klinische Wochenschrift*, 1882, xix, 221, specially prepared for The American Review of Tuberculosis, March, 1932, by Dr. and Mrs. Max Pinner. With an introduction by Dr. Allen K. Krause. Cloth, pages 48. Published by the National Tuberculosis Association, New York, 1932.

†Calcium Metabolism and Calcium Therapy. By Abraham Cantarow, M.D. Instructor in Medicine, Jefferson Medical College; in charge of Laboratory of Biochemistry, Jefferson Hospital; Assistant Physician, Philadelphia General Hospital. With a Foreword by Hobart Amory Hare, B.Sc., M.D., LL.D. Late Professor of Therapeutics, Materia Medica and Diagnosis in the Jefferson Medical College, Philadelphia. Second Edition, thoroughly revised, 252 pages, illustrated, limp binding. Lea and Febiger, Philadelphia, 1933.

‡Outline of the Cranial Nerves. By John Favill, A.B., M.D., F.A.C.P. Associate Clinical Professor of Neurology, Rush Medical College of the University of Chicago. Cloth, pages 112. The University of Chicago Press, Chicago, Ill., 1933.

Oral Spirochetes and Related Organisms in Fusio-Spirochetal Disease*

ALTHOUGH diseases which are now attributed to the fusio-spirochetal symbiosis can be traced back in medical history even to the time of Hippocrates, our attention has been more centered on them since the World War following which the dissemination of trench mouth has forced our interest in the condition. This volume discusses the biologic and cultural characteristics of oral spirochetes and fusiform bacilli, their symbiosis, and the characteristics of related organisms. Following this there is a clinical discussion of the various forms in which the infection may manifest itself including disease of the oral cavity, the bronchi, the lungs, the eyes, ears, mastoid, the nose and sinuses, the esophagus, meninges, brain, intestines, appendix, skin, and genitalia. In each case clinical characteristics, laboratory identification and therapeutic methods are presented in all necessary detail.

There has been a need for a volume such as this and it may be well recommended.

Criteria for the Classification and Diagnosis of Heart Disease†

THIS small volume, edited by the Criteria Committee of the Heart Committee of the New York Tuberculosis and Health Association, and approved by the American Heart Association, illustrates well the further application of the use of the *Standard Classified Nomenclature of Disease*, reviewed above. There will always be in the specialties need for a special system of classification of those maladies in which the physician is particularly interested. Both of these volumes have been approved by the American Heart Association and each finds its place. The volume under present review is that which would be of particular interest to those interested in cardiology. Its discussion of nomenclature in this particular field goes into much greater detail than the *Standard Nomenclature* but uses essentially the same system. In a large appendix we find very instructive chapters on radiologic diagnosis, involving orthodiagraphic tracings, and on diagnosis in the interpretation of electrocardiograms.

Outline of Preventive Medicine‡

TO MANY the title of this volume will be somewhat misleading inasmuch as it suggests the public health aspects of the subject. The volume is, however, not written from this viewpoint but rather from that of individual preventive medicine. It is a treatise prepared under the auspices of the Committee on Public Health Relations of the New York Academy of Medicine, to which 24 outstanding physicians of New York have contributed, and dealing with the early recognition and prevention of disease in the individual. Starting with a chapter on the principles and methods of the periodic health examination, it next presents a detailed discussion of the incidence of disease. There follows a section on laboratory aids in the early recognition of disease. Next comes a section on preclinical medicine and hygiene, emphasizing particularly the methods that should be applied with the individual patient in the prevention of possible future disease. Most of the remainder of the volume deals with those diseases of medicine, surgery and the specialties in which early recognition or prevention can

*Oral Spirochetes and Related Organisms in Fusio-Spirochetal Disease. By David T. Smith, A.B., M.D. Association Professor of Medicine, Duke University, School of Medicine, Durham, N. C. Formerly Bacteriologist and Pathologist to the New York State Hospital for Incipient Tuberculosis, Ray Brook, N. Y. Cloth, pages 242. The Williams and Wilkins Company, Baltimore, 1932.

†Criteria for the Classification and Diagnosis of Heart Disease. By the Criteria Committee of the Heart Committee of the New York Tuberculosis and Health Association, Inc., Joseph H. Bainton, M.D., Robert L. Levy, M.D., Arthur C. DeGraff, M.D., Harold E. B. Pardee, M.D., Chairman. Approved by the American Heart Association. Third Edition, cloth, pages 131. New York Tuberculosis and Health Association, New York, 1932.

‡Outline of Preventive Medicine. For Medical Practitioners and Students. Prepared under the auspices of The Committee on Public Health Relations, New York Academy of Medicine. 24 Contributors. Editorial Committee, Frederic E. Sondern, Chas. Gordon Heyd and E. H. L. Corwin. Second Edition revised and enlarged, cloth, pages 462. Paul B. Hoeber, Inc., New York, 1932.

be accomplished or in which treatment should be instituted at an early stage in order to prevent the ravages of the later stages. As one contributor writes, "every late case was at one time an early one."

The popularity with which this volume has been received is readily understandable.

Surgical Pathology*

THE third edition of Boyd's *Surgical Pathology* follows the general program laid out in the first two editions. While there is much on microscopic pathology, gross pathology receives ample consideration, a feature which is most essential for good practice of surgery. Illustrations are very abundant and most excellent.

The Medical Secretary†

THIS small volume is presented as a guide for doctors' secretaries and office assistants who have not had special training in this field. The advice given ranges all the way from personal appearance to the proper method of writing manuscripts for publication, and proof correction. The advice given is good and if the medical secretary were to conform to all specifications the doctor's office would run most smoothly and would be spotless. One might feel that a lot of the advice given is already obvious but when one enters some doctors' offices, one realizes that there should be a distinct need for a volume such as this that can be read and assimilated by a really ambitious secretary.

The trouble is that no matter what the qualifications of a doctor's secretary, usually his office reflects his own personality. However, it is entirely possible that a good secretary with a pride in her work and a loyalty to her employer might with the help of this small volume do wonders for a doctor who has failed to realize that the appearance and running of his office really are important from the point of view of the patient.

A third section of the book devoted to medical terminology is really a glossary of the more common medical terms. However, it would be well to emphasize that no medical secretary can get along well without a medical dictionary.

*Surgical Pathology. By William Boyd, M.D., M.R.C.P. Ed., F.R.C.P. Lond., Dipl. Psych. F.R.S.C., Professor of Pathology, University of Manitoba; Pathologist to the Winnipeg General Hospital, Winnipeg, Canada. Third Edition. Thoroughly Revised with 477 Illustrations and 13 Colored Plates, cloth, pages 866. W. B. Saunders Company, Philadelphia, and London, 1933.

†The Medical Secretary. By Minnie Genevieve Morse. Member Board of Registration, Association of Record Librarians of North America. Author of "Case Records in Small Hospitals." Cloth, pages 162. The Macmillan Company, New York, 1933.

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EDITORIAL

AIR BORNE MOLDS

THE allergist whose interest in air borne pollens takes him to the botanist for aid in the identification of weeds often finds to his surprise that the botanist knows very little of the taxonomy of the ordinary weeds and grasses. His interest both in the classroom and the field has been primarily in the brightly colored flowers and the trees. In the same way the bacteriologist has been only too well acquainted for many years with the air borne molds which are so prone to contaminate culture media, and which, indeed, he speaks of, as the weeds of the bacteriological laboratory.

When it was discovered that molds and yeasts do actually produce morbid lesions not only in the skin but in the internal organs some attention was necessarily given to this form of microorganism, but the pathologist has probably contributed as much to our knowledge in this field as has the bacteriologist. Now, since it has been found that air borne molds may be responsible for disease particularly in the allergic patient, the medical mycologist finds it desirable to acquaint himself more intimately with the weeds of the laboratory. One who has made no special studies in this field might feel that there would be considerable difficulty in finding altogether satisfactory source books for reference. As a matter of fact some very excellent reference volumes are avail-

able which deal with just those phases of the mycology of air borne molds which will be of special interest to the bacteriologist and the clinician. While there are others, some of them more detailed, two most satisfactory books are *Bacteriology* by Buchanan and Henrieci's *Molds, Yeasts, and Actinomyces*. One will rarely have occasion for more detailed information than is contained in these volumes but in such an event Stevens' *Plant Disease Fungi* provides a very complete classification, while *The Aspergilli* by Thom and Church and *The Penicillia* by Thom provides the last word in the classification of these two great groups of molds.

Mold spores are quite ubiquitous as will be vouched for by any bacteriologist, housewife or sailwright. The spore count of molds in the air varies from a matter of hundreds in the outside air to tens of thousands or even hundreds of thousands per cubic yard of air in buildings. They exist in the ground to a depth varying around four feet but are most numerous near the surface. In the earth they serve a useful purpose chiefly by breaking down cellulose into its simpler derivatives and by fixing nitrogen thereby preventing it from leaching out. Sometimes they form an almost symbiotic relationship with the roots of trees, preparing the nutritive substances in the immediate vicinity for absorption by the roots. It is said that the orchid requires its symbiotic mold for existence.

While air borne molds and yeasts are deleterious in certain industries, causing spoilage, in others they are employed to great advantage. In the Orient a mold is used to convert rice into sugar for the manufacture of alcoholic beverages. There, and in this country, *Aspergillus oryzae*, the aspergillus of rice is employed for the manufacture of a diastatic ferment, marketed as Takadiastase. The citromyces and other molds have been used for the manufacture of citric acid. The dark brown sauce used in Chinese restaurants is a product of the fermentation of *Aspergillus oryzae*. Many cheeses are ripened with molds, the most important of which are Camembert and Roquefort, which are ripened through the activity of the proteolytic ferment of a penicillium. Yeasts are employed in the fermentation of wines, beers, and in the making of bread.

Many of the air borne molds have been found to be responsible for allergic coryza and asthma, and these fortunately include most of the common ones such as the mucors, the aspergilli, the penicillia, alternaria. It is of course a logical observation that the commoner air borne molds should be responsible for this type of reaction.

The clinical bacteriologist will as time goes on in all probability develop an increasing interest not only in the pathogenic molds but in the nonpathogens mentioned above.

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—W. T. V.

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CLINICAL AND EXPERIMENTAL

THE RELATIVE INCIDENCE OF INTESTINAL PARASITES IN HOSPITAL PATIENTS IN NASHVILLE AND IN RURAL TENNESSEE*

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RECENT interest in the intestinal protozoa of man in the United States has stimulated the publication of several reports of the incidence of these parasites in cities in various parts of the country. The reports of Williamson, Kaplan and Geiger from Chicago¹ and Andrews and Paulson from Baltimore² give the incidence of all of the protozoa found, while that of Faust³ gives only the incidence of *Endamoeba histolytica*. The data for the intestinal protozoa and helminths for Nashville are given in the present report. If similar reports can be published from time to time from other cities a more accurate index of these parasites can ultimately be secured for the cities of the country as a whole.

The present report is a summary of the routine fecal examinations made in the laboratory of the Department of Preventive Medicine and Public Health of the Vanderbilt Medical School between Jan. 1, 1930, and June 30, 1932.† Of the 2,112 white and negro persons examined, 1,231 were adult medical out-patients and 881 were a miscellaneous group including pediatrics out-patients and in-patients, adult hospital patients suffering from gastrointestinal complaints and a few similar outside patients of private physicians. All of the adult out-patients and most of the other group were persons who could not afford the attendance of a private physician. They came both from

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†The author wishes to acknowledge the assistance in this work of Miss Frances Jones, technician in the department, who performed most of the examinations reported.

the city of Nashville and from the surrounding rural districts, and some from other cities. Many who resided in the city had previously lived on farms where they may have acquired their intestinal parasites. Because of this fact no attempt has been made to separate them into urban and rural groups. The results represent the incidence of these parasites from the combined urban and rural population of middle Tennessee.

The technic of fecal examination was as follows:

1. A heavy smear preparation in saline was examined for *Strongyloides* larvae and eggs of helminths.
2. A thin smear preparation in saline was examined for motile protozoa.
3. A salt-flotation test was performed for eggs of helminths.
4. About a cubic centimeter of the specimen was emulsified in tap water by shaking in a 25 c.c. test tube and filtered into a 50 c.c. centrifuge tube through "sheet-cotton" made absorbent by soaking in gasoline. The cotton was washed through with tap water up about 40 c.c., and the filtrate centrifuged at about 1,800 revolutions for one-half to one minute. The supernatant fluid was poured off and a portion of the sediment was examined in iodine eosin solution for protozoan cysts.

5. In cases where the identification of motile amebas or cysts was doubtful in fresh preparations, smears were stained with iron hematoxylin.

Most of the patients received only one fecal examination. When more than one was made it was usually done either to check the previous finding of *E. histolytica* or to follow the effect of treatment, or to make a more thorough search for the parasite in cases suspected of harboring it. This report may therefore be considered on the basis of one examination per person.

Table I shows the results of these examinations. It will be seen that *E. histolytica* was found in 63 patients or 3.0 per cent of the series. Of these 63 patients, only two were suffering from frank amebic dysentery. One other showed trophozoites which were identified as *E. histolytica* by stained

TABLE I
SUMMARY OF FECAL EXAMINATIONS ON 2,112 PATIENTS IN VANDERBILT HOSPITAL
JANUARY 1, 1930 TO JUNE 30, 1932

	1,231 FROM MEDICAL O. P. D.		881 FROM OTHER SOURCES		TOTAL	
	NO. POS.	% POS.	NO. POS.	% POS.	NO. POS.	% POS.
One or more parasites	340	27.6	302	34.3	642	30.4
<i>Endamoeba histolytica</i>	38	3.1	25	2.8	63	3.0
<i>Endamoeba coli</i>	172	14.0	97	11.0	269	12.7
<i>Endolimax nana</i>	96	7.8	57	6.5	153	7.3
<i>Iodamoeba bütschlii</i>	44	3.6	24	2.7	68	3.2
<i>Giardia lamblia</i>	34	2.8	81	9.2	115	5.5
<i>Chilomastix mesnili</i>	42	3.4	31	3.5	73	3.5
<i>Trichomonas hominis</i>	16	1.3	19	2.2	35	1.7
<i>Enteromonas hominis</i>	1	0.1	5	0.6	6	0.3
<i>Ascaris lumbricoides</i>	3	0.2	50	5.7	53	2.5
<i>Trichuris trichura</i>	3	0.2	19	2.2	22	1.0
Hookworm	11	0.9	13	1.5	24	1.1
<i>Hymenolepis nana</i>	5	0.4	19	2.2	24	1.1
<i>Strongyloides stercoralis</i>	4	0.3	10	1.1	14	0.7
<i>Enterobius vermicularis</i>	0	0.0	3	0.3	3	0.14

smear and culture, and three others showed both trophozoites and cysts. Only cysts were found in the specimens from the other 57 patients. A few of these were suffering from gastrointestinal complaints but most of them came to the hospital for trouble not apparently associated with amebic infection.

The other protozoa found in this series appeared approximately in the incidence which would be expected if the incidence of *E. histolytica* in the group is taken as a basis for comparison. The incidence of the various helminths is also in accord with what is to be expected in a combined city and rural group in this geographic region. It might be noted that *Strongyloides* larvae were found in fourteen patients, which is more than half as frequently as hookworm eggs were encountered. This indicates that *Strongyloides* is of some importance as a parasite of man in this region even in a city hospital. It will also be noted that all of the helminths as well as *Giardia* had a higher incidence in the in-patient group than in the out-patients. This is probably to be accounted for by the fact that a considerable proportion of the latter group were children, in whom the incidence of both *Giardia* and the helminths is generally higher than in adults.

Table II compares the results of the Nashville summary for protozoa with those from Chicago, Baltimore, and New Orleans. It will be seen that Baltimore seems to enjoy the lowest incidence, Chicago comes second, Nashville third, and New Orleans highest. One would expect the incidence to be progressively higher from north to south and these studies suggest that it is, with the exception of Baltimore. The patients examined by Andrews and Paulson² in Baltimore were a selected group in that they all suffered from gastrointestinal complaints. It is conceivable that such patients sometimes may lose their intestinal protozoa because of an unfavorable environment produced by other pathologic conditions in the gastrointestinal tract. It would be interesting to know whether an unselected series of hospital in-patients or out-patients in Baltimore would show a higher incidence of intestinal protozoa. In the case of New Orleans, one would expect to find a higher incidence of intestinal protozoa there than in the inland cities of the South since New Orleans is exposed to the importation of such infections from the tropics.

A comparison of the incidence of the human intestinal parasites for Vanderbilt Hospital with that for the rural population of eight of the surrounding counties and for the rural population of the State of Tennessee as a whole is given in Table III. The data for the surrounding counties and the state are taken from the recent surveys reported by Melenex, Bishop and Leathers⁴ for the protozoa and by Keller, Leathers and Bishop⁵ for the helminths. This table shows that the incidence of almost every parasite is higher in the surrounding rural area than in the Vanderbilt Hospital patients. This can partly be explained for *Giardia* and for the helminths by the fact that most of the specimens in the rural survey came from school children, in whom the incidence of these parasites is generally higher than in adults. In the case of the amebas, however, this would not apply, since their incidence has been universally found to be higher in adults than in children. The lower incidence of the amebas in the Vanderbilt Hospital patients than in the surrounding

TABLE II
HOSPITAL SURVEYS IN THE UNITED STATES FOR INTestinal PROTOZOA

AUTHORS	YEAR	PLACE	TYPE OF PATIENTS	NUMBER OF CASES	PER CENT POSITIVE FOR									NUMBER OF SPECIMENS	METHOD OF EXAMINATION	
					E. HISTOLYTICA	F. COLI	F. NANA	T. BÜCHSHEIM	D. FRAGILIS	GIARDIA	CHILOMASTIX	TRICHOMONAS	EMBADOMONAS			ENTEROMONAS
Williams, Kaplan and Geiger	1929	Chicago, Ill.	Food Handlers	1,148	2.4	19.2	2.0	5.3	--	6.5	1.9	1.8	--	--	2	Fresh specimen straight smear
Williams Kaplan and Geiger	1929	Chicago, Ill.	Normal (?) Persons, Mostly Hospital Out-Patients	380	1.1	8.1	0.8	1.7	--	1.7	1.6	1.1	--	--	1-2	Fresh specimen straight smear and concentration
Andrews and Paulson	1931	Baltimore, Md.	Hospital Out-Patients with Gastrointestinal Complaints	522	0.2	4.2	2.5	1.3	--	2.7	1.3	2.1	0.2	--	1	Straight smear. Specimens brought to clinic
Faust	1931	New Orleans, La.	Male Medical Wards Female Medical Wards Obstetric Service Ambulatory Clinic Patients (Dr. F. M. Johns)	27.2 13.1 25.2											Two-thirds received 3 or more examinations	Straight smear
Meleney	1932	Nashville, Tenn.	Hospital Out-Patients and In-Patients	2,112	3.0	12.7	7.3	3.2	0.0	5.5	3.5	1.7	--	0.3	1	Smear and concentration Specimens brought to clinic or sent from wards

TABLE III

INCIDENCE OF HUMAN INTESTINAL PARASITES IN VANDERBILT HOSPITAL PATIENTS AND IN THE RURAL POPULATION OF TENNESSEE

	PROTOZOA						HELMINTHS			
	<i>E. HISTOLYTICA</i>	<i>E. COLI</i>	<i>E. NANA</i>	<i>TOXAMOEBA</i>	<i>GIARDIA</i>	<i>CILIOMASTIX</i>	<i>ASCARIS</i>	<i>TRICHURI</i>	<i>HOOKWORM</i>	<i>HYMENOLEPIS NANA</i>
Vanderbilt Hospital	3.0	12.7	7.3	3.2	5.5	3.5	2.5	1.0	1.1	1.1
Rural population of eight surrounding counties	9.5	32.0	13.3	4.3	15.0	3.5	16.7	2.5	0.2	1.8
Rural Tennessee as a whole	11.4	32.1	11.9	4.1	14.7	2.9	27.1	7.6	6.8	2.9

rural area is probably due to the fact that the majority of the hospital patients were residents of the city, where excreta disposal is more sanitary, where flies are somewhat less abundant, and where personal hygiene is in general better than in the rural areas.

Table III also shows that the counties surrounding Nashville have an incidence of intestinal protozoa approximately the same as that for the rural population of Tennessee as a whole. In the case of the helminths, however, these counties are considerably below the incidence for the whole state due to the fact that these counties are situated mainly in the "Central Basin" of the state, where the conditions necessary for the transmission of the helminths are less favorable than in some other parts of Tennessee.

It should be remembered that the incidence percentages here reported do not represent the actual incidence in the groups studied. It is estimated that one fecal examination by the technic employed for protozoa will reveal about half of those actually harboring *E. histolytica*. On this basis the actual incidence of this parasite in the Vanderbilt Hospital patients would be about 6 per cent, that in the surrounding rural area would be about 19 per cent and that for the rural population of Tennessee as a whole would be about 23 per cent.

The higher incidence of intestinal protozoa in the rural areas is emphasized particularly in order to focus attention upon the rural population in connection with the problem of amebiasis in the United States.

Andrews and Paulson² in the report of their Baltimore study state, "The incidence of intestinal protozoa in such a civilian" (apparently meaning "city") "group as has been described, is probably more nearly representative of the incidence of intestinal protozoa in residents of the temperate zone as a whole than incidences based upon the previously mentioned surveys" (institutions, troops, mining camps, remote rural districts) "in as much as most of the population of the temperate zone now is concentrated within large cities." This statement leaves the rural population too much out of consideration. The United States Census for 1930 shows that 43.8 per cent of the population of the whole country is rural, and that even in Maryland 40.2 per cent is rural. In the portion of the country designated in the Census as "The North" 32.8 per cent, or nearly one-third of the population, is still rural, and

in the portion designated in the Census as "The South," which includes Maryland, 65.9 per cent, or nearly two-thirds, is rural. It is evident, therefore, that the rural population must still be considered important in determining the incidence of intestinal protozoa in the United States.

Again Craig⁶ in speaking of the amebiasis problem states, "It is undoubtedly true that the vast majority of infections with *Endamoeba histolytica* in this country are acquired through food handlers in public eating places" (institutions, hospitals, hotels, restaurants, etc.). "While in rural communities infection undoubtedly occurs in the home, and while this is also true to a lesser extent in the cities, it is no less true that the great source of infection in this country is the food handler in public eating places." Craig⁶ thus focuses attention on towns and cities as the location of the main problem of amebiasis in the United States. It is undoubtedly true that public food handlers are an important source of infection in cities, and the necessity for their control should not be minimized. On the other hand, the data which have been presented for Tennessee in Table III show that the incidence of amebiasis in the rural areas is three or four times as high as in the city, and it is fair to assume that this does not apply to Tennessee alone. This indicates that the conditions for transmission of the dysentery ameba are much more favorable in the rural districts than in the cities. In the matter of sewage disposal and in the protection of excreta and food from flies, American cities present on the whole much better conditions than most rural districts. Food handlers in public eating places, although they may have no adequate conception of the hygienic handling of food, usually have some degree of supervision which would at least tend to limit the frequency with which they would convey the parasite to the patrons of these places. In the poor rural home, however, where close personal contact constantly occurs, often under the most unhygienic conditions, the opportunities for transmission within the family are much greater. The mother as food handler and nurse, the children constantly playing together, and the fly with its free access to feces and food probably all contribute to the spread of the intestinal protozoa in the family. The long duration of these infections is undoubtedly responsible for their continuation and spread in new family groups by marriage. Frequent visiting between families, and the roaming habits of the fly are probably responsible in the main for transmission from house to house. It seems important, therefore, to focus attention upon the amebiasis problem in rural districts as well as in cities. It must be remembered that the adult population of cities is made up to a significant degree of persons who spent their childhood in rural districts, and that such persons often are employed as domestic servants and public food handlers. It is therefore possible that sources of infection in cities may frequently have their origin in rural areas.

In conclusion it may be said that the results of the examinations in Vanderbilt Hospital indicate that the careful routine examination of fecal specimens from hospital patients anywhere in the United States is well worth the effort in revealing the occasional presence of pathogenic animal parasites, and in discovering a pathologic condition which might not otherwise be diagnosed.

It is undoubtedly true, as Craig⁶ states, that fecal examinations are often more important for a correct diagnosis than some of the other diagnostic measures ordinarily employed as a routine at the present time.

SUMMARY

1. The results of routine fecal examinations for animal parasites in 2,112 hospital patients in Nashville, Tennessee, are reported. The incidence is somewhat higher for most of the intestinal protozoa than is reported for Chicago and Baltimore, but lower (for *E. histolytica*) than is reported for New Orleans.

2. The incidence of most of the intestinal parasites in hospital patients in Nashville is lower than for the surrounding rural area, and lower than for the rural population of Tennessee as a whole.

3. The problem of amebiasis in Tennessee is apparently more a rural problem than an urban problem, though the latter should not be ignored.

4. Careful routine fecal examinations of hospital patients should be employed much more than is the custom at the present time in the United States.

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STUDIES IN THE PHARMACOLOGY OF LOCAL ANESTHETICS

VI. COMPARISON OF METYCAINE WITH COCAINE AND PROCAINE ON EXPERIMENTAL ANIMALS

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INTRODUCTION

PRELIMINARY pharmacologic data on certain of McElvain's¹ local anesthetics, piperidine derivatives, have been reported by McElvain and Jones¹ and Rose.² Some of these compounds were submitted to Dr. W. R. Meeker of Mobile, Alabama, for comparative clinical evaluation. Dr. Meeker³ finally selected the compound gamma—(2-methyl-piperidino)—propyl benzoate hydrochloride as the compound which showed promise of a useful local anesthetic.

Since that selection considerable pharmacologic data have been reported on this compound by Coles and Rose⁴; Rose, Coles, and Thompson⁵; Coles and Rose⁶; and Rose.⁷

This article is a continuation of the pharmacologic study of this compound in comparison with cocaine and procaine, particularly, the action of gamma—(2-methyl-piperidino)—propyl benzoate hydrochloride (metycaine) on the perfused heart of frogs, on the blood pressure and respiration of anesthetized and pithed dogs, on the perfused legs of frogs (peripheral vessels), and on the sensory nerve trunk (sciatic of rabbits).

Action on the Perfused Frog Heart.—The hearts of large leopard frogs were used. The perfusion experiments were performed during the months of September and October. The Howell modification of Ringer's solution was used; that is, NaCl 0.7, CaCl₂ 0.026, HCl 0.03, the quantities referring to grams of the anhydrous salts per 100 c.c. of distilled water. This solution had a P_H of 7.2 to 7.3 (colorimetric). The temperature of the solution and of the hearts was kept at 22° C. to 24° C. during the experiments. The hearts were perfused essentially according to the method of Sollmann and Barlow⁸ under constant pressure from the vena cava, the fluid escaping from the wounds and from the inferior end of the vena cava. The perfusion was made by a heart perfusion cannula (Dr. Greene's).

Various dilutions (1:5,000, 1:10,000, 1:20,000, 1:40,000, and 1:80,000) of the local anesthetics, cocaine, procaine, and metycaine, were used. At least 3 frog hearts were used for each dilution.

Cocaine with a dilution of 1:5,000, shows a very marked decrease in rate and amplitude and inhibition of rhythm. This is also observed with dilutions of 1:10,000, 1:20,000, and 1:40,000. A dilution of 1:80,000 produces no change in amplitude and rhythm and only a slight decrease in rate (see Fig. 1).

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Procaine, with a dilution of 1:5,000, shows a distinct change in rate and amplitude and an inhibition of rhythm, although not equal to that of cocaine (1:5,000). Dilutions of 1:10,000 and 1:20,000 of procaine produce a slight decrease in amplitude and in rate without disturbance of rhythm. Dilutions of 1:40,000 and 1:80,000 of procaine produce a distinct stimulation, and an increase in amplitude and rate (see Fig. 2).

Metycaine (1:5,000) produces an auricular ventricular block. Metycaine (1:10,000, 1:20,000, and 1:30,000) produces a decrease in amplitude and a decrease in rate without disturbance of rhythm (see Fig. 3). Dilutions of

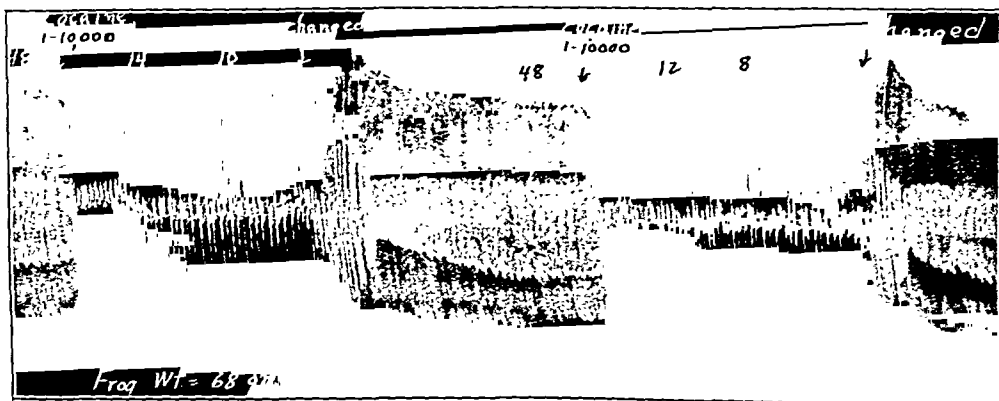


Fig. 1.—Represents a tracing of a perfused frog heart. The normal heart rate is 48. Following the perfusion of cocaine 1-10,000, the heart shows an A-V block with a marked change in rate.

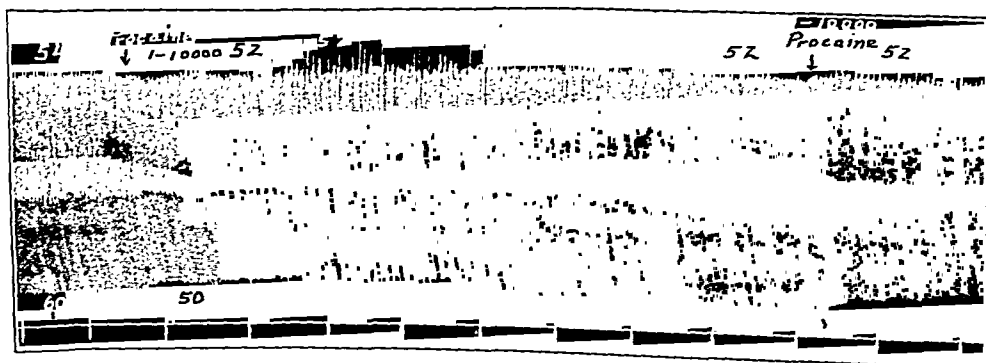


Fig. 2.—Represents a tracing of a perfused frog heart. The normal heart rate is 54. Following the perfusion of procaine 1-10,000, the heart rate is comparatively unchanged and there is only a slight decrease in amplitude.

1:40,000 and 1:80,000 produce a slight stimulation in 50 per cent of the hearts. Thus, metycaine produces less depressing symptoms on the perfused frog heart than cocaine but is more depressant than procaine.

Action on the Blood Pressure and Respiration of Anesthetized (Ether and Sodium Amytal) Dogs.—Fourteen dogs were used, 6 dogs under ether and 6 dogs under sodium amytal (40 mg. per kg. intravenously). Two dogs were pithed. The vagi were cut in some of the anesthetized experiments. Doses of 1 mg. to 10 mg. per kg. of cocaine, procaine, and metycaine were injected intravenously. In some of the experiments, alternating doses of the local anesthetics were injected in the same animal.

The effects of all three of the local anesthetics on the general circulation are partly central and partly peripheral. Cocaine in small doses (1 to 2 mg. per kg.) causes a distinct increase in blood pressure and a slower pulse rate. In moderate doses (3 to 5 mg. per kg.), the pulse is increased and a rise in blood pressure is observed. This rise in blood pressure and change in pulse rate, with small doses and moderate doses, is less in dogs with vagi cut, and practically absent in pithed dogs. Large doses (5 to 10 mg. per kg.) of cocaine produce in anesthetized and pithed dogs a very great fall of blood pressure and a weak, slow pulse.

Procaine in small doses (1 to 2 mg. per kg.) produces a very slight rise in blood pressure and very little change in pulse rate in anesthetized (ether and sodium amytal) dogs. In dogs with vagi cut or pithed, the rise is absent. The rise, which occurs after the use of small doses of cocaine, is generally higher than the rise produced by procaine. Procaine, in moderate doses (3 to 5 mg.

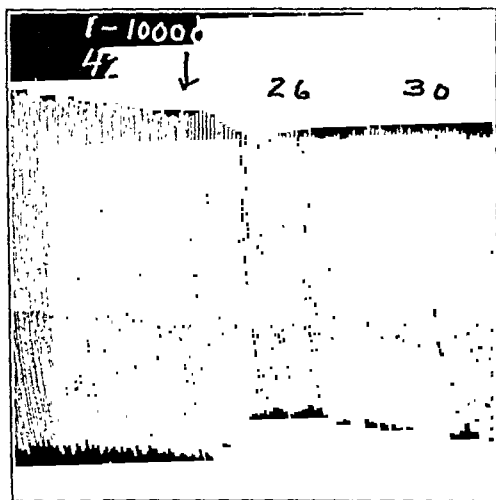


Fig. 3.—Represents a tracing of a perfused frog heart. The normal heart rate is 42. The arrow shows the change from control solution to a 1-10,000 metycaine solution. Numbers 26 and 30 represent the heart rate following the perfusion of the local anesthetic. There is a decrease in amplitude.

per kg.), generally causes no change or a drop in blood pressure and an increased pulse rate. Procaine in large doses (5 to 10 mg. per kg.) produces in experiments a distinct drop in blood pressure and a slow pulse; however, equivalent doses of cocaine produce a greater drop in blood pressure and a slower pulse.

Metycaine in small doses (1 to 2 mg. per kg.) produces either a very slight rise or no change in blood pressure, and very little change in pulse rate. With small doses (1 to 2 mg. per kg.), the fall of blood pressure predominates in anesthetized dogs (vagi cut) or pithed dogs. Metycaine in moderate doses (3 to 5 mg. per kg.) either produces no change or a slight fall in blood pressure, and a distinct increase in pulse rate. Metycaine in large doses (5 to 10 mg. per kg.) produces a fall in blood pressure and a slow, weak pulse rate. This fall of blood pressure and slow pulse rate with equivalent doses is more than with procaine and less than with cocaine.

The effects on respiration with small doses (1 to 2 mg. per kg.) of cocaine, metycaine, and procaine are acceleration, the degree of acceleration being in the order named. Moderate (3 to 5 mg. per kg.) doses of cocaine, metycaine, and procaine increase the respiration rate, but decrease the amplitude. Large doses (5 to 10 mg. per kg.) of cocaine, metycaine, and procaine cause depression of respiration, the degree of depression being in the order named.

Action on Perfused Frog Legs (Peripheral Vessels).—The perfusion method of frog legs used was essentially that of Trendelenburg's.⁹ The frog was pithed, a cannula (Greene) inserted into the abdominal aorta. The out-flow from the abdominal vein was recorded by the number of drops. The local anesthetics were perfused from a Mariotte bottle through a "Y" connection.

At least 2 or more frogs were used on each dilution. The dilutions used were 1:1,000, 1:2,500, and 1:5,000 of each local anesthetic. Epinephrine 1:40,000 dilution, and sodium nitrite 1:1,000 dilution were used to show vasoconstriction and vasodilatation after the local anesthetic was given. The results of these experiments are given in Table I.

TABLE I
PERFUSION OF FROG LEGS
(Laewen-Trendelenburg Method)*

DRUG	DILUTION	NUMBER OF FROGS	TIME OF MAXIMUM EFFECT	DECREASE (CON- STRICTION)	INCREASE (DILA- TATION)
			minutes	drops	drops
Cocaine	1: 1,000	3	15	4	—
Cocaine	1: 2,500	2	15	3	—
Cocaine	1: 5,000	3	15	—	4
Metycaine	1: 1,000	2	15	0	0
Metycaine	1: 2,500	3	15	0	0
Metycaine	1: 5,000	2	15	0	0
Procaine	1: 1,000	2	15	0	0
Procaine	1: 2,500	2	15	0	0
Procaine	1: 5,000	2	15	0	0
Sodium nitrite	1: 1,000	16	10-30	—	10
Epinephrine	1:40,000	16	10-20	35	—

*Laewen, A.: Arch. exper. Path. Pharm. 51: 416, 1904. Trendelenburg, P.: ibid. 63: 165, 1910.

As shown in Table I metycaine and procaine produce neither constriction nor dilatation. Cocaine produces constriction in dilutions of 1:1,000 and 1:2,500 and dilatation in dilutions of 1:5,000.

Action on the Sensory Nerve Trunks (Sciatic).—The method used is essentially that of Biberfeld and Pototsky and elaborated by Schmitz and Loevenhart.¹⁰ In this study, the technic of the latter was used.

As shown in Table II, a 0.75 per cent solution of metycaine produces anesthesia equivalent to a 3 per cent solution of cocaine and a 0.5 per cent solution of procaine.

Action on Smooth Muscle.—The action of metycaine, cocaine, and procaine on smooth muscle was determined on the isolated intestinal strips of rabbits, isolated bladder of rats, and isolated uteri of guinea pigs and rabbits.

On the isolated intestinal strip of rabbits, the inhibition of normal rhythm is distinct with all three local anesthetics. Metycaine in dilutions of 1:80,000 produces inhibition equivalent to cocaine 1:30,000 and procaine 1:40,000.

TABLE II
MINIMUM EFFECTIVE CONCENTRATION FOR NERVE BLOCKING (RABBIT'S SCIATIC)

DRUG	4		3		2		1		0.75		0.5		0.25	
	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS	NUMBER OF EXPERIMENTS	RESULTS
Cocaine	3	Complete anes-thesia	3	Complete anes-thesia	10	No anesthesia in 4, partial anes-thesia in 3, com-plete anesthesia in 3	3	No anes-thesia	3	No anesthesia	5	No anesthesia	5	No anesthesia
Procaine	3	Complete anes-thesia	3	Complete anes-thesia	3	Complete anes-thesia	3	Complete anes-thesia	3	Complete an-esthesia	5	Complete anes-thesia	5	Complete anes-thesia in 1, partial anesthesia in 1, no anesthesia in 3
Mety-caine	3	Complete anes-thesia	3	Complete anes-thesia	3	Complete anes-thesia	3	Complete anes-thesia	5	Complete an-esthesia in 2, partial anesthesia in 3, partial anesthesia in 2	5	Complete anes-thesia in 2, partial anesthesia in 1, no anesthesia in 2	5	No anesthesia

The action of these compounds on the isolated bladder strips of rats shows contraction and a distinct increase in rhythm, the order of their increased effectiveness in dilutions of 1:5,000 and 1:10,000 being metycaine, cocaine and procaine.

In dilutions of 1:5,000 and 1:10,000. these local anesthetics produce a distinct contraction of the isolated uterus of guinea pigs and a contraction and increased rhythm of the isolated uterus of rabbits, their effectiveness in equivalent dilutions being in the same order, metycaine, cocaine, and procaine. Thus, the action of metycaine in equivalent dilutions on smooth muscle is apparently more effective than either cocaine or procaine.

CONCLUSIONS

1. Metycaine produces less depressing symptoms on the perfused frog heart than cocaine, but is more depressant than procaine.

2. The comparative effects on the general circulation and respiration of metycaine are more like procaine than cocaine.

3. Metycaine on the peripheral vessels is more like procaine than cocaine.

4. The action of metycaine on the sensory nerve trunk (sciatic) is two-thirds as effective as procaine and more than three times as effective as cocaine.

5. Metycaine on smooth muscle is more effective than either procaine or cocaine; that is, in equivalent doses, metycaine produces more inhibition of isolated intestinal strips and more stimulation of isolated uterine or bladder strips than either cocaine or procaine.

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A FATAL CASE OF SICKLE CELL ANEMIA WITH AUTOPSY FINDINGS*

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SICKLE cell anemia, an hemolytic anemia, in which many erythrocytes have a characteristic sickle or crescent shape, was first described by Herich in 1910. Since that time the disease has been reported with increasing frequency by several observers, viz., Washburn, J. E. Cook, J. Meyers, V. R. Mason, V. P. Sydenstricker, J. G. Huch, and others.

It is a familial and hereditary disease confined largely, if not altogether, to the negro race. In those cases apparently white, investigation of the ancestry has usually suggested mixed blood. The condition is congenital, being present at birth, and is probably due to congenital defects in the spleen and blood-forming organs that result in changes in the erythrocytes which predispose them to hemolysis and phagocytosis. The anemia, when present, is due to excessive blood destruction activated, perhaps, by factors that in a normal person would be innocuous. It exists in a latent and active phase. The latent phase presents neither definite symptoms nor physical signs and is recognized only by special blood examination. The active phase presents definite symptoms and physical signs and the characteristic sickling may be seen on an ordinarily stained blood smear.

A number of cases have been reported in recent years, probably the largest series by Sydenstricker. In view of the fact that case reports with autopsy findings are very few, we feel justified in presenting this case with autopsy report.

CASE REPORT

CASE 1.—Colored male, aged twenty-one, was admitted to the hospital on Nov. 27, 1931. At this time he complained of swelling of the entire body, shortness of breath, weakness and vertigo. The onset of the present illness was six months prior to admission, at which time he noticed edema of the feet and legs which gradually extended to the abdomen, face, and arms. Dizziness, weakness, dyspnea, palpitation, and headaches had been troublesome. Occasionally there was moderate epigastric pain brought on by drinking water or eating. On a previous admission to the hospital, September, 1931, a diagnosis of chronic nephritis was made. At this time he was anemic, red cell count 2,030,000, but the phenomenon of sickling was not noted. He remained in the hospital four weeks with subsidence of the edema and improvement of his general condition.

Past History.—He had gonorrhea in 1929 without complications, diphtheria in 1927, pneumonia in 1922, mumps and measles when a small child, frequent attacks of tonsillitis, and several ulcers on the anterior surfaces of both legs six or eight years ago. His mother died at the age of forty-one of Bright's disease. There was no history of blood dyscrasias in the family.

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Physical Examination.—He was a fairly well-developed and nourished colored male with generalized edema and moderate dyspnea. There was an icteric tinge to the sclerae and the tonsils were hypertrophied and cryptic. A few moist râles were heard over the bases of the lungs. There was a systolic murmur heard best at the apex of the heart, not transmitted, and there was no apparent enlargement of the organ. Blood pressure was 150 systolic and 70 diastolic. The abdomen contained free fluid and the liver was palpable two finger-breadths below the costal margin. The spleen was not palpable. There were several scars on the anterior surfaces of the legs about the middle third.

The patient remained in the hospital continuously from Nov. 27, 1931, to Feb. 2, 1932. Treatment was largely of a supportive nature. A suitable donor for blood transfusion could not be obtained either from his family or those who volunteered. Liver extract, ventriculin with iron and iron alone had no effect on the anemia. He ran a low grade temperature up to the last week, then 103.4° F. which we attributed to an exacerbation of the nephritis and bronchopneumonia. He died Feb. 2, 1932.

The report of the laboratory work, made at intervals during his stay in the hospital, is given in Table I.

Necropsy.—The body was that of a well-developed colored male, aged twenty-one, with generalized edema. The pupils were equal, round, and measured 5 mm. in diameter. There was slight jaundice of the sclerae and marked pallor of the conjunctivae. The teeth and gums were in good condition, tongue normal in appearance and consistence and the buccal mucosa was pale. The edema of the upper extremities was quite marked from the elbows downward, including the wrists and hands. The abdomen bulged. The pubic hair was normal in quantity and distribution. The thighs and legs were equal and the skin, which pitted on pressure, was tense and shiny. The posterior cervical and inguinal lymph nodes were palpable. There were several scars on the anterior surfaces of the legs.

Abdomen.—The subcutaneous adipose tissue had a maximum thickness of 5 cm. in the midline anteriorly and presented a pale, gelatinous appearance due to the accumulation of fluid which was given up freely on compression. Several liters of lemon colored, slightly turbid fluid were found in the peritoneal cavity. The peritoneum was smooth and was tinged yellow in color. The liver extended the breadth of four fingers below the costal margin in the midclavicular line, right. The appendix was small, retrocecal, and the serous covering was pale and smooth. There was marked edema of the mesentery and the fat in the omentum, which was rather scant, was a peculiar pale brownish color, and was waterlogged. The mesenteric lymph nodes were enlarged and sectioned surfaces were pale pink. The liver weighed 2250 gm., edges were rounded, and the capsular surface was smooth. Externally it was bluish red in color. Sectioned surfaces bulged slightly and were generally red or brownish red with small patches of yellow here and there. Surfaces were bathed with thin, watery blood. The spleen was found as an elongate, wedge-shaped body lying along the gastrolinal ligament. Its inferior extremity was in contact with the tail of the pancreas while the superior pole did not quite extend to the stomach. Its position was well toward the median line and much lower than the position occupied by a normal organ. It measured 5 and 10 mm. respectively at the upper and lower extremities in width and its greatest thickness was 8 mm. It was 4 cm. long. There were deep wrinkles in the thick, gray capsule. Sectioned surfaces were brownish in color, dry, slightly retracted, and presented a sievelike appearance. The pancreas was normal in size, firm, and the lobules were conspicuous. Surfaces were pink in color. The suprarenals were normal in size, the pigmented zone normal in color and width, and the medullae were pinkish in color. The kidneys were about equal in size and weighed together 430 gm. There was considerable yellowish red fatty tissue about each organ. The capsules stripped easily in cutting resistance and surfaces bulged slightly. The cortices measured from 5 to 9 mm. minute, bulging red points. The pyramids were slightly swollen and, like the cortices and outer surfaces, mottled. The pelvic fat was not excessive and the pelvic lining was smooth and pale. The ureters were free from abnormalities, normal in size, and the lining was

TABLE I
LABORATORY DATA

URINE			
DATE	11/27/31	12/15/31	1/21/32
Specific gravity	1.013	1.010	1.010
Reaction	Alk.	Neut.	Acid
Albumin	4-plus	1-plus	2-plus
Sugar	None	None	None
Acetone	None	None	None
Casts	Occ. hyaline	None	Hy. F. & G. Gr.
Pus cells	Many	Few	Loaded
Epithelial cells	Bladder	Occ. sq.	Sq. & blad.
Crystals	Phos.	Phos.	
Amorphous sed.	Heavy	Small amt.	
Bacteria	Mod. No.	Few	Many
Erythrocytes	Many	None	Few
BLOOD			
DATE	11/30/31	12/15/31	2/2/32
Total white	13,350	14,509	17,950
Neutrophiles	58	59	76
Lymphocytes	40	28	17
Eosinophiles	2	5	
Basophiles		1	
Large mon.		7	7
Total R. B. C.	1,700,000	1,940,000	1,230,000
Hemoglobin	33%	30%	20%
Color index	0.9		
Platelets	260,100	224,100	
Coag. time	2 min.		
Bleed. time	6 min.		
Nucleated reds	Many	13	
Poikilocytosis	Marked	Marked	
Anisocytosis	Marked	Marked	
Achromia	Marked	Marked	
Polychromasia	Marked	Marked	
Sickling	Many		
Reticulocytes	6.5%		
Wassermann	Neg.		
Kahn	Neg.		
Total protein	4.05	6.75	
N. P. N.	33.3	26.4	
Cholesterol		298.4	
Albumin	2.06		
Globulin	1.90		
Urea		11.9	
Icteric index		30.3	
Fragility test	No hemol. in dilutions from 0.5 to 0.28		
Basal metab.	-13	-9	1/13 -7
Gastric analysis	Free HCl 13; Total acidity 51		

pale. The *urinary bladder* wall measured $1\frac{1}{2}$ cm. in thickness and there were large, clear bullae involving the lining of the organ. On section, these latter were found to represent submucous accumulations of serous fluid. The *prostate* and prostatic urethra showed nothing noteworthy. The *stomach* was normal in size. The rugae were quite conspicuous, and there was diffuse bluish red or dark red color in the lining. There was edema of all parts of the wall of the small and large intestines as well as passive congestion of the lining.

Thorax.—Fluid of the same character as noted in the peritoneal cavity was present in both pleural cavities (amount estimated, 2 liters). The pericardial sac contained 750 c.c. of clear, straw-colored fluid. The *lungs* were small. They were dark red at the bases and posteriorly and were mottled gray and black at the apices and anteriorly. There were old interlobar and apical adhesions on the right. The cutting resistance was increased and sur-

faces were brownish red in color. Rusty, frothy fluid could be expressed from cut surfaces of both organs. There were no areas of consolidation and anthracosis was moderate but diffuse. The main bronchi contained frothy fluid and their lining was pale. The tracheo-bronchial lymph nodes were anthracotic. The heart weighed 410 gm. and was slightly ovoid in shape. There was a milk patch on the anterior surface of the right ventricle measuring 2 cm. in diameter. The epicardial fat was not excessive. There were no gross organic lesions of any of the orifices. The valve leaflets, cusps, and the mural endocardium were tinged

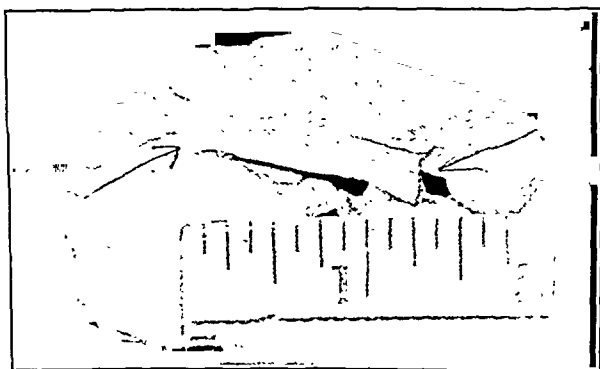


Fig. 1.—Spleen. Arrows point to the extremities of the organ. This represents full size except for $\frac{1}{2}$ cm. removed for section. A portion of the pancreas is above and a section of the stomach wall below.

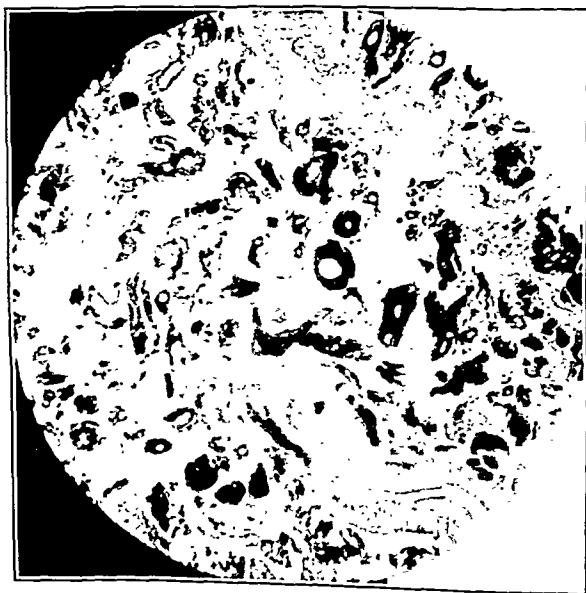


Fig. 2.—Section from spleen showing fibrosis of the pulp and necrotic malpighian bodies (dark areas). The thickened capsule is noted as infoldings (low power).

yellow. The myocardium was brownish red in color and fairly firm in consistence. The maximum thickness of the left ventricular wall at the base was $2\frac{1}{2}$ cm. Rather conspicuous scars were noted in the left ventricular wall at its base. The lining of the coronary arteries and aorta was smooth and pale.

The convolutions of the brain were moderately flattened, and there was an excess of fluid in the subarachnoid space.

The bone marrow in the upper thirds of the tibiae and humeri were red.

Microscopic Examination.—Tissues were fixed in formaldehyde and paraffin sections stained with hematoxylin and eosin.

Heart.—There was moderate perivascular fibrosis which radiated between the adjacent muscle bundles. In addition, there were focal areas of degeneration with beginning connective tissue replacement. Relatively large areas of scarring which extended out to the epicardium were noted in some of the sections. There was diffuse engorgement of the small vessels. In places the nuclei were quite large.

Lungs.—There was an increase in the peribronchial and finer connective tissue about the alveoli. The alveolar septa were quite thick, due both to the feature mentioned above (fibrosis) and engorgement of the capillaries. Red cells were present in many of the alveoli as well as "heart failure cells." Many of the alveoli contained pink staining serous coagulum. The interstitial element was heavily laden in places with black pigment.

Aorta.—The aorta presented nothing noteworthy.

Spleen.—The capsule was thick and dense, and there were convolutions which extended deeply into the organ, almost meeting similar infoldings of the capsule from the opposite

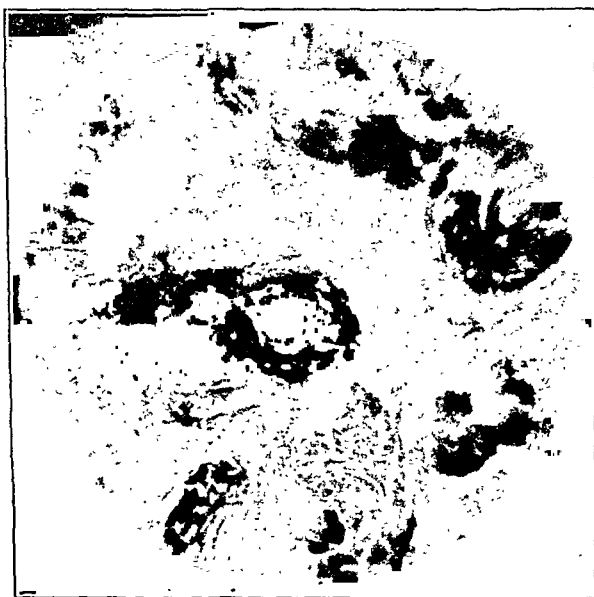


Fig. 3.—Section from spleen (high power).

side. Trabeculae were not identified. The pulp was completely replaced by dense, relatively acellular but somewhat loosely arranged connective tissue. This latter separated dark staining oval or oblong areas in which clear-cut cellular structure was conspicuously absent. These appeared to be composed of the necrotic remains of the malpighian corpuscles. In the centers of most of the dark staining areas was a less dense oval space containing either homogeneous pale staining material, old erythrocytes or dense connective tissue. Surrounding these was a darker zone of varying width composed of débris of necrotic cells and, in not a few instances, old erythrocytes and brown pigment.

Liver.—The capsule was slightly thickened. The central veins and adjacent portions of the sinusoids were engorged with erythrocytes, while the sinusoids at the periphery of the lobules showed this feature less conspicuously. The cell cords were relatively narrowed. There was diffuse hemachromatosis and cloudy swelling of the hepatic cells as well as fatty change in places. There was a varying amount of fibrosis, lymphocytic and, to a less extent, eosinophilic infiltration about the portal canals. In some instances fibrous strands radiated for some distance replacing the degenerated parenchyma. In such areas regeneration of bile capillaries was noted.

Kidneys.—The glomeruli showed a variety of changes which represented stages in the same progressive process. Proliferation of the subcapsular cells with formation of crescents or circles which in many instances showed fusion, fibrosis and hyalinization was a conspicuous feature. Many of the glomerular tufts were small and in some instances presented as small fibrous or hyalinized balls. Adhesion of the tuft of capillaries to the thickened capsule of Bowman was present in many places. Interstitial fibrosis and infiltration with lymphocytes, plasma cells, and an occasional eosinophile, particularly around the glomeruli showing advanced change, were widespread in the cortex. The tubules were dilated in many instances and contained a serous coagulum or hyaline casts. The tubular epithelium showed cloudy swelling, fatty degeneration and necrosis, not diffuse. The capillaries were generally engorged and the glomeruli, normal in appearance, completely filled the subcapsular space in most instances.

Pancreas.—Other than diffuse congestion and cloudy swelling, this organ showed nothing noteworthy.

Suprarenals.—Postmortem change.

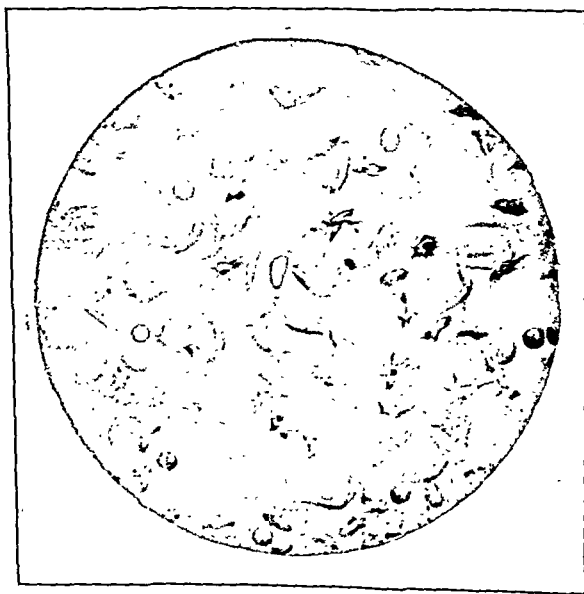


Fig. 4.—Wet preparation of blood showing sickling.

Lymph nodes.—The mesenteric and inguinal nodes showed diffuse fibrosis. There was an accumulation of large, pale macrophages as well as an occasional eosinophile and neutrophile in some of the sinuses. A few eosinophiles were present diffusely in the cortex and pulp cords.

Summary of Gross and Microscopic Diagnoses.—Chronic passive congestion, edema and anthracosis of lungs; chronic pleuritis, right; chronic interstitial pneumonia; chronic fibrous myocarditis with moderate hypertrophy of heart; milk patch on anterior surface, right ventricle; cloudy swelling, hemachromatosis, fatty change, passive hyperemia and slight perilobular fibrosis of liver; necrosis and atrophy of spleen; passive hyperemia and cloudy swelling of pancreas; subacute and chronic productive capsular glomerulonephritis; chronic lymphadenitis; hypertrophy of bone marrow; chronic hypertrophic gastritis; anasarca; hydrothorax, bilateral; hydropericardium; ascites; jaundice; anemia.

DISCUSSION

This patient presented the characteristic findings of sickle cell anemia with an associated nephritis. The blood examinations showed the characteristic anemia, sickling, and leucocytosis. In addition, a decrease in the fragility of the erythrocytes was noted, no hemolysis occurring in dilutions which began with 0.5 and ended with 0.28. In five latent cases, detected by routine examination of wet preparations on 137 negro patients, delayed fragility was also noted, hemolysis beginning in dilution 0.38 and incomplete in dilution 0.28.

The characteristic pathology appears to be confined to the spleen and bone marrow. The findings in neither have been uniform which, perhaps, is best interpreted by assuming that different stages of a progressive process were encountered. There may be enlargement or atrophy of the spleen. Congenital malformation of the sinuses with free escape of blood into the pulp and the formation of blood pools around the malpighian bodies are probably characteristic of the latent phase and the early or intermediate stages of the active form. Marked atrophy of the organ with complete fibrosis of the pulp and necrosis of the few remaining malpighian bodies, as in our case, probably indicate long duration of active disease.

The bone marrow picture is that of chronic infection or injury with compensatory hyperplasia. Here again, the findings have not been constant. In our case there was simple hyperplasia of the marrow, while in Graham's case, there was chronic productive inflammation with centrifugal involvement of the bone. Chronic osteomyelitis, periostitis, leg ulcers, and even osteitis have been observed radiologically and at necropsy, but there may be fatal cases with atrophy of the spleen and the terminal complications such as nephritis and pneumonia which show none of these.

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BLOOD CATALASE IN HEART DISEASE*

1. PRELIMINARY REPORT

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IN 1863 Schoenbein¹ described the catalytic effect of some substance in vegetable and animal tissues which liberated oxygen from a solution of hydrogen dioxide. The activity of this principle could be diminished by the addition of weak acids, heat, and other anticatalytic agents and because of its evident biologic nature he considered it to be an enzyme and called it catalase. From close observation of the type of reaction it has been claimed² that this enzyme in tissues is relatively inactive but that in the first phase of its reaction with the dioxide it becomes an active catalase. Furthermore, its activity is in direct proportion to the hydrogen ion concentration of the solution, rising up to a certain point and in inverse proportion to the temperature within certain limits, becoming less active as the temperature rises. The optimum temperature for the reaction is about 10° C., but temperatures up to 40° C. cause little, if any, inactivation whereas at 58° C. about 25 per cent catalytic action is lost.^{4, 5, 6, 7}

In testing for catalase different grades and brands of commercial hydrogen dioxide solution have given varying yields of oxygen with the same tissue but the errors could be eliminated by neutralizing the solution with sodium hydroxide.⁸ Margolis and his coworkers⁹ found that high concentrations of hydrogen dioxide destroyed catalase in tissues and drew attention to the necessity of proper dilution.

The function of tissue catalase has been a subject of considerable dispute and its true value is still unappreciated. The majority of authors feel and have shown by more or less conclusive deductions that it is an important metabolic factor, particularly in intracellular oxidative processes. Burge⁹ found that the catalase content of tissues is dependent on or proportionate to the metabolism and varies with the respiratory exchange. A high protein diet, as one would expect from its specific dynamic action, has been shown to cause an increase in catalase content,^{10, 11} and after alcohol ingestion a similar rise has been observed.¹⁴ Experimentally a fall in tissue catalase has been produced by starvation,¹² castration, thyroidectomy,¹³ and to a less extent by pancreatotomy.

Clinically, changes in catalase content have been recorded in many diseases. In primary anemia, a rise has been observed whereas in cachectic diseases,¹⁵ cancer, hemolytic anemias, febrile diseases and pulmonary tuberculosis,¹⁶ there is a distinct fall. Its association with the metabolic rate has been noted further in many experimental and clinical observations as in the re-

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ported associated variations of both in the same individual during different parts of the day,¹⁷ the occurrence of less tissue catalase in young kittens than adult cats,¹⁸ and the 70 per cent increase in the blood during a mountain sojourn as compared with an increase of 38 per cent in the erythrocytes.¹⁹ Varying favorably with the differences in metabolic rates, Alexeeff, et al.,²⁰ have found that the catalase content of male blood exceeds that of the female by 10 per cent. Robben,²¹ however, challenges this metabolism theory of catalase function and shows that catalase added to muscle exercised no effect on the respiratory exchange. Moreover, Stehle^b found that catalase content of blood is directly proportionate to the concentration of red corpuscles, and is not related to biologic oxidative processes. Furthermore, in a large number of experiments on protein feeding, he failed to produce an increase in catalase except in one case. In order of quantity catalase has been found and measured in the following tissues: blood, kidney, liver, spleen, lungs, heart, brain, and muscles.²² Blood serum contains no catalase,²³ corpuscles suspended in isotonic solution fail to give the typical reaction with hydrogen dioxide, but laked erythrocytes liberate oxygen from the reagent,²⁴ the intensity of reaction varying with the number present.²⁵

Catalase determinations have been reported in human organs both in health and disease. Diseased kidney has a decreased value, the content being less with the more extensive pathology.^{26, 27} The rôle of erythrocytes is emphasized in the study of a pneumonia lung. During the stage of red hepatization, the catalase activity is increased over that of a normal lung and in a hemorrhagic infarct it is enormously increased. On the other hand, during gray hepatization, the pulmonary catalase is decreased. During ether or chloroform anesthesia, the catalase content of all tissues is decreased,²⁸ asphyxia having the same effect.²⁶ There is no appreciable change in diabetes, senility, eclampsia,²³ and there is no relation between change of heart rate and blood catalase.²⁹ Sollmann³⁰ investigated the catalase activity of the oral mucous membrane and found that there was no diagnostic change in the various forms of sore throat.

In this study, the authors make no attempt to explain the rôle of blood catalase, but wish to point out the changes in heart disease and their diagnostic value.

TECHNIC

In our work, several methods were tried, many being complicated. The set-up which proved to be the simplest to operate and the most efficient is that described by Hawk and Bergheim³¹ with one minor modification by us. Most of the pathologic cases were seen at the Cardiac Clinic of the Boston City Hospital between ten and eleven o'clock in the morning, and all the blood specimens were taken at that hour in the order of the patient's appearance at the clinic and without knowing the diagnosis. Each patient was questioned about the content of his breakfast; those having had meat or eggs were tested on the following day when protein was eliminated from the meal. Complete blood examinations were done when indicated. After the oral temperature and pulse rate were recorded, blood was drawn into a special calibrated white

blood corpuscle diluting pipette up to the five mark from a puncture wound in the ear lobe. The pipette was then filled with distilled water and the contents transferred to a small test tube, several distilled water washings of the pipette being added to the laked blood. This was then corked and stored with the other morning's samples in the laboratory at 16° C. for twenty-four hours.

To 85 c.c. of a 3.5 per cent solution of hydrogen dioxide,³² 15 c.c. N/10 sodium hydroxide solution were added, both reagents having been stored at the same temperature as the blood. The above mixture was made up each day the tests were done and prepared afresh for each ten blood samples.

The diluted laked blood was introduced into a 250 c.c. distilling flask and 10 c.c. of neutral hydrogen peroxide solution added from a Johnson modified burette, the flask being rotated until a reaction became apparent, and the oxygen liberated collected over water by displacement in a graduated cylinder. After an arbitrary time limit of five minutes, from the moment the hydrogen dioxide solution was introduced, the number of cubic centimeters oxygen liberated was read directly from the cylinder.

One hundred thirty-six blood samples from the same number of apparently healthy individuals were examined in this way and the normal range was found to be 10 to 15 c.c. oxygen. One-fifth of that number and several of the pathologic cases listed in the table were checked on different days, the figures agreeing within 5 per cent.

Four hundred and twenty-nine cases were studied and the amounts of oxygen liberated by the individual bloods carefully checked. When a blood caused a 19 c.c. yield, it was repeated to be certain that it was not a borderline case and its proper classification ascertained. The average normal figure was found to be about 15 c.c., thus putting all the normal cases in the 10-20 group.

In the last two groups of Table I, we find practically all the cases of rheumatic and scarlatinal endocarditis. There were 116 cases of mitral and aortic valvular disease of rheumatic etiology in the third group, a few of which were decompensated. All these were felt to be inactive affections as determined by the general clinical picture of each case and observation over a period varying from weeks to years. Seven others in this third group were found to be active, some at first examination and others after close study, the average yield being 29 c.c. in these. In the final group 18 cases of active rheumatic endocarditis gave the typical high result, two having been thought to be sub-acute bacterial endocarditis but the diagnosis was never finally established.

The three septicopyemia cases fall into the first two groups and are sharply differentiated from the rheumatic endocarditis of the latter groups.

Thirteen cases of scarlet fever are listed in the lower range, all having no organic heart complications. Six other cases, known to have a heart lesion arising as a complication of the fever fall into the class of rheumatic endocarditis, two being definitely active. The results in these cases are of particular interest when we consider the newer theory of relation between rheumatic fever, chorea, tonsillitis, and scarlatina. In 1899 McCollum³³ reported three cases of endocarditis as a complication of scarlet fever in a series of 1000 consecutive cases. Broadbent³⁴ reported endocarditis in 0.58 per cent of scarlet

TABLE I
DISEASES AND NUMBER OF CASES STUDIED, CLASSIFIED ACCORDING TO OXYGEN YIELD

	0-10	11-20	21-30	OVER 30
Normal		135		
Aortitis, syphilitic	1	3		
Aneurism			1	
Myxedema		3		
Hyperthyroidism		2		
Colloid goiter		3		
Diabetes mellitus		8		
Infectious arthritis		24	1	
Arthritis deformans		14		
Leucemia, myelogenous	2			
Leucemia, lymphatic	1			
Epilepsy			1	
Psychoneurosis	7			
Nephrolithiasis	1			
Hodgkins disease	2			
Bronchopneumonia			1	
Angina pectoris		2		
Hypertension		16		
Nonvalvular heart disease	1	22		
Functional murmurs	7	11		
Diphtheria myocarditis	2	4		
Scarlet fever				
(a) Normal hearts	1	12		
(b) With active endocarditis			4	2
Rheumatic heart disease				
(a) Decompensated	1			
(b) Compensated and decompensated			116	
(c) Active			7	18
Gonococcal acute endocarditis		1		
Staphylococcal acute endocarditis	2			
Total cases		429		

fever complications while Reinhard³⁵ found it in only one case of his series. Nobecourt³⁶ believes that the same etiologic agent in scarlet fever, rheumatic fever, chorea, and tonsillitis cause similar heart lesions, and Fahr,³⁷ from his pathologic studies, affirms his opinion of the general similarity of heart affections in scarlet fever and rheumatic infection. Sutherland,³⁸ on the other hand, believes that an attack of scarlet fever sensitizes the endocardium in individuals susceptible to rheumatic infection and that such infection is latent in those cases becoming active in the presence of scarlatina. Swift³⁹ has classified the heart complications of scarlet fever as follows:

1. Toxic, similar to those complicating diphtheria and occurring in the first week of the disease. They are due to the action of the disease. They are due to the action of the toxin on the myocardium.
2. Septicopyemic, malignant and rare.
3. Allergic, which occurs in the third and fourth week of the disease and is due to an acquired hypersensitiveness to the streptococcus or its toxin.

Despite the fact that the number of cases is small, the blood reaction in scarlatinal endocarditis closely resembles that of rheumatic etiology and further study is warranted.

In five instances the catalase content of blood proved to be of no diagnostic value. The first case was that of a decompensated mitral stenosis in which the yield was only 10 c.c. The second, an epileptic, yielded 26 c.c., an

infectious arthritis 21 c.c., aortic aneurysm 24.5 c.c., and a bronchopneumonia 38 c.c. The last case was that of a prolonged migratory bronchopneumonia in which there were no heart complications. These determinations were repeated with identical results.

In the early part of our work, we attempted to correlate our catalase values with the valve affected, intensity of physical signs, degree of dyspnea and other symptoms, grade of hypertrophy, but so far have found no relation to these factors.

At the present, our knowledge consists in the recognition of normal catalase values in human blood from 0-20 c.c. A value of 21-30 indicates valvular disease, the final diagnosis being dependent on the entire clinical and laboratory picture. At about 26, the lesion is probably active and over 30 certainly active. This was illustrated in the case of a medical student, twenty-six years of age, who first came under observation in October, 1931. At that time he was examined routinely on admission to the school and a tachycardia (rate 110) noted. He complained of nervousness but otherwise felt well. Ten years before he had severe pains in his legs and was obliged to stay in bed for two weeks. From that time until his admission to medical school he had frequent sore throats but no return of joint pains and he had been frequently examined in school and college. Aside from the rapid pulse and cryptic tonsils the examination was normal. A month later a basal metabolism rate was advised because of the nervousness and persistent tachycardia. Two tests were performed, both being normal.

The patient was seen by one of the authors in December, 1931, and early signs of heart disease were noted. The pulse rate was 96, rhythm regular, heart not enlarged to percussion, but there was a short blowing systolic murmur at the apex, intensified by exercise and transmitted to the axilla. The first sound at the apex was short and of a snapping character and the second sound in the pulmonic area was reduplicated. A blood catalase test was performed, the result being 44. He was advised to enter a hospital but refused and was not seen again until Jan. 20, 1932. Then he complained of weakness, pallor, palpitation, and precordial pain. Examination of the heart revealed a long crescendo presystolic murmur and snapping first sound at the mitral area. The pulmonic second sound was greatly accentuated. Apical and radial pulse rates were 126. An electrocardiogram showed a P-R interval of 0.20 second and a QRS complex of 0.08 second duration. The blood catalase finding was 41. The patient was hospitalized and given large doses of salicylates together with complete rest. An orthodiagram revealed no heart enlargement.

The blood catalase quantity dropped to 28 c.c. oxygen yield over a period of two weeks and the pulse rate to 86. Tonsillectomy was performed on the fifteenth day and the patient discharged to his home on the sixteenth day after entry. At that time P-R interval was 0.16 second and the duration of the QRS complex 0.06 second.

The authors were unable to get in touch with the patient again until March 20, 1932, at which time he was obviously an ambulatory cardiac. He complained of intermittent attacks of fever, palpitation, dyspnea, precordial

pain and weakness. Physical examination of his heart revealed a rate of 100, enlargement to percussion, a long harsh diastolic murmur and short first sound at the mitral area.

The results of a blood catalase test were 40.5 c.c. and this dropped to 30 c.c. after a week's rest at his home and in May, 1932, he was seen again with the same symptoms and signs and a catalase value over 40 c.c.

In this particular case the authors feel that an earlier diagnosis might have been made by a catalase test when his tachycardia was first observed. The fact that a relatively short period of bed rest caused symptomatic and actual improvement as shown by the change in catalase content of his blood should suggest that a longer treatment would have arrested the lesion and probably brought the catalase value below 26.

The authors were fortunate to obtain permission to study the blood reaction and use the record of two patients who came to necropsy.

CASE 1.—This twenty-three-year-old white female entered the Boston City Hospital Jan. 12, 1931, complaining of easy fatigue ability, shortness of breath and swelling of the ankles of several years' duration. There was a history of rheumatic fever several years before and she had had a crop of red, tender nodules on her legs two months before admission, which persisted for five weeks. The patient was kept in bed for three weeks, during which time the temperature was normal and she was sent home with a diagnosis of rheumatic heart disease. On March 3, 1931, she was readmitted to the hospital. Fever was the only complaint at this time and had persisted since February 21, when she had a mild sore throat, chilly sensations, and pains and swelling of the wrists and hands. Examination revealed a fairly well-developed and nourished, young female, not acutely ill. The eyes, ears, nose, and throat were normal. According to the record, physical examination of the heart revealed that the point of maximum intensity was in the fifth interspace outside of the midclavicular line. There was a loud systolic murmur at the apex which was transmitted to the axilla; the first sound at the mitral area was loud and snapping and the second sound at the pulmonic area accentuated. The blood pressure was 110/70. The lungs and abdomen were normal, and there was no swelling of the joints. Examination of the nervous system revealed no abnormalities.

Laboratory Examinations.—Urine and sputums were normal. The white count was 15,600 on admission. Chest plates showed a mass to the right of the superior mediastinum, and an enlarged heart with a mitral deformity. Repeated blood cultures were negative except for one in which there was a growth of *Streptococcus viridans*. Late in her illness, the authors determined the catalase content of her blood and found it to be 18.5. This result, in view of the above studies, was not consistent with a diagnosis of subacute bacterial endocarditis. The catalase reactions repeatedly remained normal.

Two weeks after admission, the chart showed a wild, swinging temperature ranging from 97° to 104°, until demise Jan. 19, 1932. Many roentgenograms of the chest were taken, were interpreted variously, and did not aid in clarifying the situation. Except for small, red, tender, skin nodules cultures from which showed *Staphylococcus aureus* which kept recurring and the development of enlarged swollen and tender knees, the condition of the patient did not change much over a period of several months. Death occurred suddenly.

Clinical Diagnosis.—Subacute bacterial endocarditis, acute dilatation.

Postmortem Examination.—The body was that of a poorly developed, poorly nourished white female. There was marked swelling of the first phalanx of the middle finger on each hand. Both knee joints and the right ankle joint were markedly enlarged. Over the anterior surface of the right thigh were several round, white scars about 1.5 cm. in diameter.

Pleural Cavities.—The left pleural cavity was negative. The right pleural cavity in the region of the lower lobe was obliterated. The pleuras were thickened and on cross-section varied in thickness from 8 mm. to 2 cm. Patches of soft, yellow material, 0.3 to 1 cm.

in diameter were scattered through the dense gray tissue representing the pleuras. In the region of the eighth rib at its point of juncture with the vertebral column, situated behind the pleura was a pocket of thick, yellow fluid 4 cm. by 2 cm. The ends of the ribs in this region were eroded.

Pericardial Cavity.—Negative.

Heart.—Weight 410 gm. There was a moderate degree of enlargement. The muscularis was dark red and firm with no gross areas of scarring or softening. The endocardium was smooth and *all valves were negative*. Both coronary arteries were patent throughout. There were no congenital anomalies of the heart or of the pulmonary artery or aorta.

Spleen.—Weight 220 gm. This organ was enlarged. The capsule was thin and wrinkled. Section showed a dark reddish purple parenchyma of soft consistence. The cut surface scraped with ease.

Joints.—On opening the right knee joint, a quantity of thick, gray fluid containing many yellowish gray flakes, was discharged. There was about 200 c.c. of this fluid. The synovial surfaces were fibrin coated. The synovia was soft, red, and thickened. It was closely adherent to the cartilage over the medial condyle of the femur. The semilunar cartilages could not be identified. The joint cartilage was smooth and glistening, except over the area of attachment to the synovia.

ANATOMICAL DIAGNOSES

Old pleurisy with abscess formation.

Retropleural abscess.

Purulent arthritis.

Cardiac hypertrophy.

Chronic passive congestion of the liver.

Enlargement of the kidneys.

In this case the low blood catalase coefficient was the only finding inconsistent with a clinically typical picture of subacute bacterial endocarditis, and the only antimortem finding consistent with the absence of an endocarditis at autopsy.

CASE 2.—J. G., a forty-seven-year-old male restaurateur was admitted to the Boston City Hospital in November, 1931, complaining of febrile attacks of five months' duration. Symptoms of fever, weakness, and loss of weight came on after a tonsillectomy in June, 1931. He had lost about sixty pounds of his weight during this time. Physical examination was entirely negative aside from the intermittent fever. Roentgenograms of chest, gastrointestinal system and skeleton were normal. The Kahn, Widal, agglutination tests for tularemia, red corpuscle count, and hemoglobin tests were normal. Blood cultures and repeated searches for blood and intestinal parasites were negative. There was a persistent leucopenia, the count varying from 2,700 to 4,400. The differential count was normal. Blood catalase determinations averaged 16 thus excluding the possible diagnosis of rheumatic heart disease or subacute bacterial endocarditis. Quinine by mouth and arsphenamine intravenously were finally tried empirically. The patient improved and was discharged during a remission in January, 1932.

The fever recurred and he was admitted to another hospital for two weeks where no diagnosis was made. He reentered the Boston City Hospital in March, 1932, complaining of fever for three weeks, vomiting and diarrhea following an injection of arsphenamine. Physical examination at this time revealed further loss of weight, an enlarged liver and spleen and areas of brownish pigmentation on chest and back. He had convulsions on the third day which became more frequent and severe. The patient died on the fourth day after reentry.

Clinical Diagnosis.—Unexplained fever; ? subacute bacterial endocarditis.

Anatomical Diagnosis.—Lymphoblastoma involving liver, spleen, bronchial and hypergastric nodes. Focal necrosis in liver.

SUMMARY

We offer herewith:

1. Some considerations relative to the catalase reaction.
2. Our technic and standardization.
3. Results in 429 cases.
4. Suggestions as to the relationship of scarlet fever endocarditis to rheumatic heart disease.
5. In a small series (6 cases) of diphtheria with definite heart damage, the catalase was normal.
6. Three specific cases: (a) Early diagnosis and course of activity by the catalase test. (b) Case clinically resembling rheumatic heart disease with subacute bacterial endocarditis (including one positive blood culture, *Streptococcus viridans*). Catalase was normal. (c) Doubtful case clinically. Negative evidence by means of test.

CONCLUSIONS

1. The determination of blood catalase is of value in making a diagnosis of rheumatic heart disease especially in doubtful cases.
2. It is of help in determining the activity or inactivity of a valve lesion.
3. Study of the blood catalase in active rheumatic endocarditis will aid in determining the length of time required for treatment.

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A STUDY OF BLOOD CALCIUM AFTER THE ADMINISTRATION OF SODIUM OXALATE TO NORMAL AND THYROPARATHYROID-ECTOMIZED CATS*

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SINCE oxalate forms an insoluble compound with calcium, a number of investigators were led to study its effects on calcium in the body, and, although their results were obtained in experiments on different animals and by different methods, they all agreed that the administration of soluble salts of oxalic acid was accompanied by deprivation of calcium from the tissues. According to Sarvonat and Roubier,¹ a considerable loss occurred in the soft parts as well as in the skeleton when oxalate was given to guinea pigs, in sufficient amounts, for several days. Luithlen² reported the elimination of large quantities of calcium in the urine of rabbits after the administration of oxalate, and Gross³ found that the calcium content of dog's blood, after subcutaneous injections, was reduced 50 per cent, while Vines⁴ obtained similar results in experiments on rabbits after the administration of ammonium oxalate. McCrea⁵ reported that blood calcium was likewise decreased in cats that received sodium oxalate, and Gley and Kokas,⁶ in studies on rabbits and cats, and more recently Chiao Tsai and Fong Yen Hsu,⁷ in experiments on dogs, furnished additional data that oxalate lowered the calcium level in the blood. With the exception of Vines and the Chinese investigators, no attempt has, as far as we know, been made to determine the speed of recovery, nor have any observations been reported on the effect of the amount, the frequency or the method of, administration of oxalate on the concentration of blood calcium. As we found that a relation existed between calcium depletion, produced by oxalate, and pharmacologic reactions,⁸ a study of its action, with special reference to those factors, was considered particularly advisable. The present investigation was, therefore, undertaken and observations were made on the changes in blood calcium produced after the subcutaneous or intravenous administration of different amounts of sodium oxalate, attention being directed also to the duration of the effect and to the influence of smaller doses when given for a period of several days. Owing to the rôle which the parathyroids play in calcium metabolism, experiments were also carried out on the effects on blood calcium produced by oxalate after their removal, for the results under these conditions might throw some light on those obtained when it is given to normal animals.

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Methods.—Cats, which were kept on a uniform diet of salmon, bread and milk with water ad libidum, were the subjects of the experiments. As indicated above, sodium oxalate was given subcutaneously and also intravenously, in which case urethane anesthesia was sometimes employed. The doses administered by intravenous injections were 10 mg. sodium oxalate per kilo about ten minutes apart, and the amounts administered subcutaneously were 20 mg. per kilo every twenty or thirty minutes. Blood calcium was determined by the Kramer Tisdall⁹ method, the blood for analysis being usually drawn from the heart, but it was sometimes obtained from the femoral artery, instead. In all cases, calcium was determined before as well as after oxalate, and in thyroparathyroidectomized animals analyses for calcium were made before the operation and also about ten to thirty minutes before the injection of oxalate was begun. In some experiments more than one analysis were made in the interval between extirpation of the gland and the administration of oxalate. The operations, for the removal of the thyroid and the parathyroid, were carried out under proper aseptic precautions, with ether as the anesthetic. Oxalate was given intravenously, usually within two days, but a longer interval was sometimes allowed to elapse.

Results.—Although depression of the calcium level of the blood by oxalate, was produced by subcutaneous as well as by intravenous injections, the effect, caused by the two methods of administration, varied considerably. One hour after the subcutaneous injection of a dose of 20 mg. sodium oxalate per kilo,

TABLE I
SERUM CALCIUM, AFTER SUBCUTANEOUS INJECTIONS OF SODIUM OXALATE

CAT	SEX	WT. KILO.	BEFORE OXALATE	AFTER 20 MG. OXALATE PER KILO DAILY						
			MG. CA PER 100 C.C. SERUM	MG. CA PER 100 C.C. SERUM, AMT. DECREASE, TIME AFTER INJECTION						
				INJECTION NO.						
				I	II	III	IV	V	VI	VII
7	female	2.3	9.8	10 2% 1 hr.	9.3 6% 1 hr.	8.6 13% 1½ hr.	8.0 19% 25½ hr.	—	—	—
8	female	2.4	9.7	9.7 0% 1½ hr.	7.9 19% 1 hr.	7.3 25% 1½ hr.	—	—	—	—
10	female	2.3	10.0	9.6 5% 1 hr.	9.3 8% 1½ hr.	9.2 9 1 hr.	8.6 15% 1 hr. 5% 24 hr.	8.8 21% 1 hr.	—	10.0 1% 30 hr.
11	female	2.6	10.8	9.9 8% 1 hr.	9.9 8% 1½ hr.	9.1 16% 1 hr.	9.1 16% 1 hr. 11% 24 hr.	8.5 21% 1 hr.	10.3 4% 30½ hr.	—
12	female	2.6	10.5	10.4 1% 1 hr.	9.7 7% 1½ hr.	9.7 7% 1 hr.	9.5 9% 1 hr. 6% 24½ hr.	8.6 18% 1 hr.	10.4 0.1% 30½ hr.	—

practically no effect on blood calcium was observed in three out of five experiments, while the remaining two showed a decline of 5 and 8 per cent (Table I). The results were quite different when this dose was repeated for several days. While very marked changes in the concentration of calcium after the second dose were seldom observed, the fall being 6 to 8 per cent in 4 experiments and 19 per cent in only 1, the decrease of calcium in the blood was much greater after subsequent injections. The maximum diminution was 16 and 25 per cent after the third, 15 to 19 per cent when the fourth dose was given, 18 and 21 per cent after the fifth. We also observed that the depletion was progressive, the decrease of calcium reaching a maximum one hour after the third, fourth, and fifth injections, in different experiments, and, in one case, this was noticed even twenty-five and one-half hours after the fourth dose. Illustrative of the cumulative effect of oxalate was one experiment, in which no change was observed after the first dose, but the drop in the concentration of calcium was 19 per cent after the second, and 25 per cent after the third injection, and another, in which the first dose was likewise without demonstrable disturbance of blood calcium, the decrease was 6, 13, and 19 per cent after successive injections. Replacement apparently occurred as well as loss, as about twenty-four hours after the injection the calcium content of the blood was appreciably greater than it was one hour after the same amount was given, and in 3 experiments the effect of oxalate on blood calcium was negligible at the end of thirty hours (see Table I).

When the amount of oxalate was increased to 40 mg. per kilo, which was given subcutaneously in divided doses, and the blood was drawn one-half to one hour later, the fall of serum calcium was 2.5, 5.1, and 13 per cent in 3 experiments, but, in 2 others, it was 25 and 26 per cent. The results were not greatly different, in some experiments, with 80 mg. oxalate per kilo, as the decrease of calcium, half an hour after the injection, was 11 to 20 per cent in 4 experiments, but in 2 it was 36 and 37 per cent. The calcium content of the blood, four hours after the administration of these amounts of oxalate, failed to show any change from that previously observed, in some experiments. A distinct improvement occurred, however, in others, calcium showing a rise of 8 to 13 per cent above the previous level. On the other hand, a still further depression sometimes occurred, for in one experiment, with 40 mg. oxalate per kilo, the decrease of calcium was 25 per cent one hour after the injection and 32 per cent three and one-half hours later. Analysis of the blood within twenty-three to twenty-nine hours, after the administration of 40 mg. oxalate per kilo, indicated that the blood calcium regained its normal level in some cases and was 12 and 16 per cent below in 2 others. The changes were even more irregular after 80 mg. oxalate per kilo, the decrease of blood calcium in 2 experiments being greater on the day following than it was a few hours after the injection, and in one of these, depression of the calcium level continued as the concentration, fifty hours after oxalate was given, was less than on the previous day. In 4 other experiments, in which the fall of blood calcium half an hour after the administration of this amount of oxalate was 16 to 20 per cent, no significant change occurred in the course of about twenty-

four hours. But in 2 experiments in which, as mentioned above, pronounced depression of the calcium level was produced, the decrease amounting to 36 and 37 per cent half an hour after the injection of oxalate, the diminution of blood calcium was only 6 and 9 per cent about twenty-one hours later. To sum up the results obtained in this series of experiments, it may be stated that complete recovery was observed in about twenty-four hours only when 40 mg. oxalate per kilo were given, while in experiments with double this amount, the normal level was not restored until a period of forty-five to fifty-one hours after the injections was allowed to elapse.

TABLE II*

REPLACEMENT OF SERUM CALCIUM AFTER SUBCUTANEOUS INJECTIONS OF SODIUM OXALATE

CAT	SEX	WT. KILO	MG. CA PER 100 C.C. SERUM BEFORE OXALATE	OXALATE MG. PER KILO.	MG. CA PER 100 C.C. SERUM, AMT. DECREASE, TIME AFTER OXALATE			
4	female	2.2	10.7	40	9.3 13% 1 hr.	9.3 5.6% 4 hr.	11.0 slight increase 23 hr.	—
6	male	3.5	10.2	40	7.5 26% 1 hr.	8.4 17% 4 hr.	8.9 12% 24 hr.	—
9	female	3.7	9.8	40	7.4 25% 1 hr.	6.7 32% 4 hr. 20 min.	8.2 16% 29 hr. 45 min.	—
14	female	2.2	10.8	80	8.6 20% ½ hr.	8.8 19% 4 hr.	9.3 14% 27 hr.	—
3	female	3.0	10.6	80	6.7 37% ½ hr.	7.5 29% 5 hr.	9.6 9% 21 hr.	10.6 0% 45 hr.
5	female	2.1	10.1	80	6.5 36% ½ hr.	7.8 23% 4 hr.	9.5 6% 22 hr.	—
1	male	2.2	11.3	80	10.0 11% ½ hr.	9.1 19.4% 6½ hr.	8.8 22.1% 23½ hr.	7.8 30.9% 50½ hr.
2	female	2.75	11.5	80	9.3 20% ½ hr.	9.4 18.2% 6½ hr.	9.6 16.5% 23½ hr.	11.3 1.7% 50½ hr.

*Tables II, IV, and V contain only some of the experiments given under the respective headings.

Wide variations in the decrease of blood calcium also occurred after intravenous injections even when the same amounts of oxalate were given. Six to ten minutes after the injection of 20 mg. sodium oxalate per kilo, the diminution of blood calcium was 10 to 28 per cent in 4 experiments, and, exceptionally, these differences were greatly increased, for while we found that, in one experiment, the fall, after one hour, amounted to 3.6 only, and the concentration of serum calcium in another was decreased 6 per cent, in a third it was 43 per cent. It may be remarked, in this connection, that the blood cal-

practically no effect on blood calcium was observed in three out of five experiments, while the remaining two showed a decline of 5 and 8 per cent (Table I). The results were quite different when this dose was repeated for several days. While very marked changes in the concentration of calcium after the second dose were seldom observed, the fall being 6 to 8 per cent in 4 experiments and 19 per cent in only 1, the decrease of calcium in the blood was much greater after subsequent injections. The maximum diminution was 16 and 25 per cent after the third, 15 to 19 per cent when the fourth dose was given, 18 and 21 per cent after the fifth. We also observed that the depletion was progressive, the decrease of calcium reaching a maximum one hour after the third, fourth, and fifth injections, in different experiments, and, in one case, this was noticed even twenty-five and one-half hours after the fourth dose. Illustrative of the cumulative effect of oxalate was one experiment, in which no change was observed after the first dose, but the drop in the concentration of calcium was 19 per cent after the second, and 25 per cent after the third injection, and another, in which the first dose was likewise without demonstrable disturbance of blood calcium, the decrease was 6, 13, and 19 per cent after successive injections. Replacement apparently occurred as well as loss, as about twenty-four hours after the injection the calcium content of the blood was appreciably greater than it was one hour after the same amount was given, and in 3 experiments the effect of oxalate on blood calcium was negligible at the end of thirty hours (see Table I).

When the amount of oxalate was increased to 40 mg. per kilo, which was given subcutaneously in divided doses, and the blood was drawn one-half to one hour later, the fall of serum calcium was 2.5, 5.1, and 13 per cent in 3 experiments, but, in 2 others, it was 25 and 26 per cent. The results were not greatly different, in some experiments, with 80 mg. oxalate per kilo, as the decrease of calcium, half an hour after the injection, was 11 to 20 per cent in 4 experiments, but in 2 it was 36 and 37 per cent. The calcium content of the blood, four hours after the administration of these amounts of oxalate, failed to show any change from that previously observed, in some experiments. A distinct improvement occurred, however, in others, calcium showing a rise of 8 to 13 per cent above the previous level. On the other hand, a still further depression sometimes occurred, for in one experiment, with 40 mg. oxalate per kilo, the decrease of calcium was 25 per cent one hour after the injection and 32 per cent three and one-half hours later. Analysis of the blood within twenty-three to twenty-nine hours, after the administration of 40 mg. oxalate per kilo, indicated that the blood calcium regained its normal level in some cases and was 12 and 16 per cent below in 2 others. The changes were even more irregular after 80 mg. oxalate per kilo, the decrease of blood calcium in 2 experiments being greater on the day following than it was a few hours after the injection, and in one of these, depression of the calcium level continued as the concentration, fifty hours after oxalate was given, was less than on the previous day. In 4 other experiments, in which the fall of blood calcium half an hour after the administration of this amount of oxalate was 16 to 20 per cent, no significant change occurred in the course of about twenty-

TABLE IV

SERUM CALCIUM AFTER INTRAVENOUS INJECTIONS OF SODIUM OXALATE INTO NORMAL CATS

CAT	SEX	WT. KILO	MG. CA PER 100 C.C SERUM, AMT. DECREASE					SOD. OXALATE, MG. PER KILO
			BEFORE OXALATE	TIME AFTER OXALATE				
				3-10 MIN.	1-1½ HR.	4 HR.	24 HR.	
55	female	3.0	10.3	8.4 19%	8.2 21%	—	9.9 5%	30
1R	—	2.9	9.9	8.4 20%	—	9.1 13%	—	
4R	female	3.7	10.1	9.2 11%	9.0 13%	9.7 6%	10.3 —	
5R	male	3.1	10.4	8.8 15%	8.1 22%	9.3 10%	9.4 9%	
13	—	—	—	6.6	6.6	7.3 10.6%	—	40
5A	male	3.5	10.4	6.4 38%	7.1 31%	7.7 26%	—	
61	female	2.2	11.0	4.2 62%	—	—	—	80
57	female	3.1	10.6	2.2 79%	—	—	—	120

A distinct tendency to recovery was indicated, however, by the results of the analysis of the blood obtained four hours after oxalate was injected. The decrease of blood calcium at this time, after 30 mg. oxalate per kilo, was only 6 to 13 per cent, the average being 8.4 per cent, whereas the reduction, ten minutes after this dose was given, varied between 11 to 25 per cent with an average of 18 per cent. In experiments with 40 mg. oxalate per kilo, the reduction of blood calcium at the end of two and one-half hours to five and one-half hours varied approximately between 21 and 27 per cent. With the exception of one experiment, in which the decrease at the end of five hours and forty minutes amounted to 8 per cent only, the average was about 25 per cent, representing a gain of 6 per cent over that observed six to seventeen minutes after the injection. The increase was thus 3 to 4 per cent greater after 30 mg. oxalate per kilo, during a period of about three to three and one-half hours. A further, and much greater, rise in the concentration of calcium occurred during the following period, of about twenty hours. In 2 experiments with 30 mg. oxalate per kilo, the blood calcium was only 5 and 6 per cent below normal, while in a third, restoration was complete. That the recovery was distinctly slower after 40 mg. oxalate per kilo was also shown in this case, as in one experiment the level of blood calcium was still 14 per cent below that of the control period.

When larger doses of oxalate were given intravenously, the depression of the calcium level was, of course, more pronounced than in the experiments with smaller amounts, but the relative decrease was not always proportional to the quantity injected. The loss was 8.1 mg. in one experiment one minute after the injection of 70 mg. oxalate per kilo, in another this was only 6.8 mg., though the dose of oxalate was 80 mg. per kilo, the percentile fall amounting to 66 and 62 per cent respectively. The loss was not greatly different when the dose of oxalate was still further increased. Three and five minutes after the injection of 110 and 120 mg. oxalate per kilo, the loss of calcium was 7.5

cium, before oxalate, was almost constant, being 10.0 to 10.3 mg. per 100 c.c. serum, while in one cat only it was 9.6 mg. Illustrative protocols, in brief, are given in Table III.

TABLE III

SERUM CALCIUM AFTER INTRAVENOUS INJECTIONS OF 20 MG. SODIUM OXALATE PER KILO

Cat 6, Male, Wt. 2.8 Kilo				
TIME	TREATMENT	MG. Ca PER 100 C.C. SERUM	PER CENT DECREASES OF Ca	REMARKS
11:03 A.M.	11 c.c. blood drawn	10.3	18.0%	15 min. after second in- jection of oxalate
11:16 A.M.	2.8 c.c. 1% Na ₂ C ₂ O ₄			
11:16 A.M.	2.8 c.c. 1% Na ₂ C ₂ O ₄			
11:31 A.M.	12 c.c. blood drawn	8.3		
Cat 16, Wt. 5.8 Kilo				
11:53 A.M.	10 c.c. blood drawn	9.6	12.1%	6 min. after second in- jection of oxalate
11:57 A.M.	3.8 c.c. 1% Na ₂ C ₂ O ₄			
12:10 P.M.	3.8 c.c. 1% Na ₂ C ₂ O ₄			
12:16 P.M.	11 c.c. blood drawn	8.4		
Cat 62, Female, Wt. 2.0 Kilo				
10:30 A.M.	10 c.c. blood drawn	10.1	28.0%	10 min. after second in- jection of oxalate
10:40 A.M.	2 c.c. 1% Na ₂ C ₂ O ₄			
10:50 A.M.	2 c.c. 1% Na ₂ C ₂ O ₄			
11:00 A.M.	10 c.c. blood drawn immediately after death	7.3		

This inequality in the reduction of blood calcium, produced by the same amount of oxalate, is not easy to explain but, according to Percival and Stewart¹⁰ and to Liu,^{11, 12} the quantity of parathyroid hormone in the circulation determines the amount of diffusible calcium, and since oxalate combines with this fraction of the total calcium, the difference in the extent of depletion may thus be accounted for. A wide divergence in the effect produced was also observed when larger doses of oxalate were injected, for, ten minutes after the administration of 30 mg. per kilo, calcium was decreased 10 to 25 per cent in 7 experiments and, as in the case of the preceding experiments, the two extremes were met with, a reduction of 3 per cent only in one and 48 per cent in another. When the dose was raised to 40 mg. per kilo, the range was 25 to 49 per cent in 10 experiments, while in one case the reduction was only 12 per cent. Notwithstanding, however, the fluctuations in the calcium level observed after oxalate in these experiments, it was, nevertheless, found that the average decrease rose with the size of the dose.

In the absence of the complicating factors of the absorption of oxalate, the replacement of calcium in these experiments could be studied with greater advantage than after subcutaneous injections. There was scarcely any indication of recovery for about one and one-half hours after it was administered, as examination of the blood obtained at the end of this time, after 30 and 40 mg. per kilo, failed to show any appreciable change in the concentration of calcium from that observed when blood was drawn a few minutes after the intravenous injection of oxalate. On the contrary, a further decrease of calcium could sometimes be noticed since we found, in one experiment (5R Table IV), that the concentration fell 15 per cent below normal after ten minutes and 22 per cent fifty minutes later.

crease of calcium averaged 2.0 mg., while the relative decrease was 18 per cent. After the same amount of oxalate per kilo was given to thyroparathyroidectomized cats, the average decline was only 0.45 mg. calcium per 100 c.c. with an average of 13 per cent for the relative decrease. The fall of the calcium level was much greater when 40 mg. oxalate per kilo were given, the average decrease in normal and thyroparathyroidectomized animals being respectively 3.5 and 0.8 mg., while the relative decrease was 33 per cent in one and 17.3 per cent in the other. It may be remarked in this connection that these data do not include abnormal deviations from the above levels. The decrease of calcium after removal of the thyroids and parathyroids, in one experiment with 30 mg. oxalate per kilo, was negligible, while in another, after 40 mg. oxalate per kilo, the loss was 2.5 mg., whereas in the rest this varied between 0.7 and 1.0 mg. The loss of calcium was also much greater in normal than in thyroparathyroidectomized animals when the doses were considerably increased. After 70 to 120 mg. oxalate per kilo, injected into normal cats, the average decrease was 7.7 mg. calcium per 100 c.c. serum, and the relative decrease was 70 per cent. In thyroparathyroidectomized animals, that received 80 to 140 mg. oxalate per kilo, the absolute reduction averaged 3.5 mg. and the percentile fall was 60 per cent. Of interest also are the data presented above as showing that the difference in the decrease of calcium in normal and parathyroidectomized animals is much greater after small than after large doses, the ratios being about 4:1 and 2:1 respectively.

The duration of the effect of oxalate was also studied in cats after removal of the thyroid and parathyroids, but the observations were confined to experiments with doses of 30 and 40 mg. of sodium oxalate per kilo. The level of blood calcium, about one hour after the injection, was not greatly different from that observed ten minutes after the salt was given. In one experiment only was the difference appreciable as the decrease was 13 per cent ten minutes after oxalate and 23 per cent one hour later. The decline in the concentration of calcium at the end of four hours showed that it was only 8 per cent more in one and 9 per cent more in another, while in a third the decrease was, on the contrary, 4 per cent less than it was three hours previously. As the diminution, during the same period in another experiment amounted to 21 per cent, the earlier attempt at recovery may be regarded as exceptional. Although improvement occurred on the following day, the amounts of calcium were 0.9 to 1.3 mg. below those before oxalate, while the relative differences were 12 to 21 per cent. When the effect is compared with that produced by the same amounts of oxalate in normal cats, it becomes evident that the recovery is retarded after extirpation of the parathyroids.

DISCUSSION

The picture of the blood calcium in normal cats, before the intravenous administration of oxalate, presented a striking contrast to that observed after its injection. Whereas the concentration of calcium was practically constant or showed small differences only, before treatment, a wide range of variation occurred after oxalate, even when the same amount, in proportion to body

and 8.4 mg., the percentile reduction amounting to 73 and 79 respectively. As calcium depletion was 18, 31, and 66 per cent, after doses of 30, 40, and 70 mg. respectively, it shows that the depression increased with, but was out of proportion to, the size of the dose. This does not hold, however, in the case of still larger doses, for the average diminution of calcium after 110 and 120 mg. oxalate per kilo was about 76 per cent, which is considerably below the figure demanded if the decrease were proportional to these amounts. There is apparently a maximum dose of oxalate above which the decrease of calcium proceeds very slowly.

The blood calcium when oxalate was given after removal of the thyroid and parathyroids presented quite a different picture from that observed after it was administered to normal animals. The difference may be readily appreciated by comparing Tables IV and V, and is further emphasized in Table VI, in which the average losses of calcium in normal and in thyroparathyroidectomized cats are reported.

TABLE V

SERUM CALCIUM AFTER INTRAVENOUS INJECTIONS OF SODIUM OXALATE INTO THYROPARATHYROIDECTOMIZED CATS

CAT	SEX	WT. KILO	MG. CA PER 100 C.C. SERUM, AMT. DECREASE						OXALATE MG. PER KILO
			NORMAL	AFTER THYROPARATHYROIDECTOMY					
				BEFORE OXALATE	10 MIN. AFTER OXALATE	1 HR. AFTER OXALATE	4 HR. AFTER OXALATE	23 HR. AFTER OXALATE	
10	male	3.4	10.3	7.5 27%	6.6 12%	6.9 8%	6.2 17%	6.6 12%	30
7	female	2.9	11.1	7.5 32%	6.5 13%	6.6 12%	6.0 20%	7.2 17%	
6	male	2.8	9.4	5.2 45%	4.5 14%	4.6 14%	3.4 35%	4.1 2%	40
19	female	2.8	—	5.8	3.6 40%	—	—	—	80
22	female	3.0	10.6	6.3 41%	2.0 67%	—	—	—	140

As shown in Table VI, the absolute as well as the relative decrease of calcium was considerably greater in normal animals than after removal of the thyroid and parathyroids. It will be noticed that, about ten minutes after the injection of 30 mg. sodium oxalate per kilo into normal cats, the absolute de-

TABLE VI

REDUCTION OF SERUM CALCIUM AFTER OXALATE. EXPERIMENTS ON NORMAL (N) AND THYROPARATHYROIDECTOMIZED (T) CATS

OXALATE PER KILO	AVERAGE DECREASE OF CALCIUM, MG.	PER CENT DECREASE OF CALCIUM	REMARKS
N 30	2.0	18	Calcium determined in blood obtained about ten minutes after the intravenous injection of oxalate
T 30	0.45	13	
N 40	3.5	33	
T 40	0.8	17.3	
N 70-120	7.7	70	
T 80-140	3.5	60	

fore, justify the conclusion that the function of the parathyroids form an important element and may, indeed, be the principal factor in the determination of the effects of oxalate on the changes in blood calcium.

SUMMARY

1. The subcutaneous injections of oxalate produced the following changes in serum calcium: A single dose of 20 mg. per kilo was without any effect, but a progressive decrease of calcium occurred when this amount was given daily over a period of several days. A dose of 40 mg. oxalate per kilo caused a very moderate decrease of calcium within half an hour after the injection, and was much greater, about four or five times, after an interval of one hour. Signs of recovery were observed about four hours after the injection, and the normal level of calcium was regained in about twenty-four hours. When the dose was increased to 80 mg. per kilo, depletion of calcium half an hour after the injection was considerably greater than after 40 mg. doses. A moderate rise was observed four to five hours later, and although improvement was marked, in most cases, on the following day, no change or even a further decline of calcium was observed in some cases. Complete recovery occurred about two days after the injection.

2. The effects of oxalate after intravenous injections. An average decrease of 20 per cent occurred about ten minutes after 20 and 30 mg. per kilo, and a reduction of approximately 33 per cent ten to seventeen minutes after 40 mg. per kilo. A moderate amount of recovery was observed about four to five hours after the injection of 30 mg. oxalate per kilo, which was much less when 40 mg. per kilo were given. Although the rise in serum calcium was considerable after 30 and 40 mg. per kilo on the day after the injection, it was much less after the latter amount. The reduction of the absolute amount of calcium after 70 to 120 mg. oxalate per kilogram was much greater, but the relative decrease was less than after smaller doses.

3. After extirpation of the thyroid and parathyroids, the average decrease of calcium caused by the intravenous injection of oxalate, of 30 and 40 mg. per kilo, was about a fourth of that produced by the same amounts in normal animals, but the amount of reduction by large doses, the maximum of which exceeded that given to normal cats, was less than half of that produced in normal cats.

4. The results were discussed and the explanation offered that the effects on blood calcium after small, repeated doses and after large single doses, of oxalate injected subcutaneously, were due to the injury which it caused to the parathyroid, and that variation in the amount of diffusible calcium, due to different amounts of parathyroid hormone, was the main, if not the only, factor in determining the wide range of reduction of calcium in normal animals after the intravenous injection of oxalate. The small decrease of blood calcium after the administration of sodium oxalate to thyroparathyroidectomized cats was attributed to the low content of diffusible calcium.

weight, had been administered. As the changes in blood calcium, when oxalate was given after removal of the parathyroids, manifested, on the contrary, only small variations, it is evident that a close relationship existed between parathyroid function and the extent of calcium depletion produced by oxalate in normal animals. The observations of Percival and Stewart as well as those of Liu, referred to above, have shown that the amount of diffusible calcium varied with the quantity of parathyroid hormone, while Trendelenburg and Goebel¹³ and later Salvesen and Linder¹⁴ found that it was the diffusible rather than the inactive calcium which was decreased in tetania parathyreo-priva, which would explain the difference we observed, since it is this fraction which is, in all probability, acted upon by oxalate. As the hormone would be greatly reduced after extirpation of the parathyroids, a correspondingly lower decrease in calcium, produced by oxalate, should occur, as was indeed the case. This would be contradicted, however, by the large reduction of calcium, after doses of 80 to 140 mg. per kilo, which, as shown in Table VI, averaged 3.5 mg. Of interest in this connection is the suggestion of Salvesen and Linder¹⁴ that the nondiffusible is transformed into diffusible calcium, in order to maintain the equilibrium between the two forms. That the diffusible calcium determined the extent of reduction, even after large amounts of oxalate were injected, was made highly probable by the greater decrease in normal than in thyroparathyroidectomized animals.

That the changes in blood calcium after subcutaneous injections of oxalate are also related to parathyroid function is suggested by the following observations. In experiments with small doses given daily, over a period of several days, it was found that the first injection was practically without any effect. Since the same amount given intravenously produced a considerable fall of blood calcium, the difference must be due to absorption. As oxalate when given subcutaneously would enter the circulation gradually, the small quantity of blood calcium thus bound is probably replaced by the activity of the parathyroid hormone, and the normal level is thus maintained. The reduction of calcium, when the injection of the same amounts of oxalate was continued for several days, may be explained if the toxicity and cumulative effect of the latter are taken into consideration. It has been shown by Salant and Swanson,¹⁵ Gates and Meltzer,¹⁶ Gates,¹⁷ and later by Gross,³ that oxalate is of itself toxic, and Salant and Swanson pointed out, in experiments on rabbits, that its effect was also cumulative, which was corroborated by us in experiments on cats. It is highly probable, therefore, that oxalate is injurious to the parathyroids, and that their function is depressed, thus interfering with the restoration of calcium. Injury to the parathyroids might likewise be a causative agent in the slow recovery sometimes observed after larger doses of oxalate, since the increased deficiency of hormone, by the greater damage to the gland, would necessarily lessen the speed of replacement and thus delay the return of blood calcium to its normal concentration. The same mechanism would likewise account for the progressive decline in the amount of blood calcium after the injection of large doses of oxalate, namely, failure to replace the calcium bound by oxalate. The results, presented above, there-

FIVE HUNDRED AND NINETEEN VOGE BROMINE TESTS OF URINE FOR PREGNANCY*

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EXAMINATION of 519 specimens has shown that the Voge bromine test is far from a satisfactory test for the diagnosis or exclusion of pregnancy. Positive results were obtained in but 63 per cent of the pregnancy urines and negative results in only 84 per cent of the nonpregnancy urines.

Voge¹ in 1929 proposed a bromine test for pregnancy based on the principle which he states as follows: "In view of the fact that a connection has been established between the elaborated product of the pituitary and compounds of B-iminazolyI (histamine, histidin and tethelin), it was thought advisable to test for the presence of this substance in the urine of pregnant women." He applied Knoop's² bromine test for histamine to the urine in the following way: To 2½ c.c. of urine in a test tube add 1 c.c. of bromine water. Heat to boiling.

In a positive test the color changes to pink in an alkaline urine or brownish pink in an acid urine, the color fading rapidly. In a negative test the color remains unaltered. The bromine water is prepared by diluting one part of 2 per cent bromine water with two parts of tap water.

Voge reported the results of his test with 60 specimens, the diagnoses being established by Aschheim-Zondek tests on the same specimens of urine. As will be seen in Table I he obtained excellent results in this small series of cases. Dodds³ in 1930 and Siddal et al.⁴ in 1931 reported their results with the test. Their percentage of agreement was much lower than that of Voge as will be seen in Table I in which are tabulated the results of the three reports, as well as our results in 519 specimens.

TABLE I

AUTHOR	TOTAL SPECIMENS	PREG-NANCY	POSITIVE		FALSE NEGATIVE		NOT PREG-NANT	NEGATIVE		FALSE POSITIVE	
			NUM-BER	PER CENT	NUM-BER	PER CENT		NUM-BER	PER CENT	NUM-BER	PER CENT
Voge	60	25	24	96	1	4	35				
Dodds	305	229	171	74.3	58	25.7	76	33	94	2	6
Siddal								66	86.9	10	13.1
et al.	260	36	27	75	9	25	224				
Young	519*	131	82	63	49	37	388	207	92	17	8
								325	84	63	16
Total:	1144	421	304	77	117	23	723	630	89	92	12.7

*All but a few were two tube tests.

Dodds draws the following conclusions: "It is apparent that the test is not pathognomonic of pregnancy. The error of 25.7 per cent in the antenatal

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CONCLUSION

Examination of 519 specimens has shown that the Voge bromine test is far from a satisfactory test for the diagnosis or exclusion of pregnancy. Positive results were obtained in but 63 per cent of the pregnancy urines and negative results in only 84 per cent of the nonpregnancy urines.

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PHYTOPHARMACOLOGY OF STOMACH WASHINGS IN VARIOUS
DIGESTIVE DISORDERS AND PERNICIOUS ANEMIA*

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DURING the past ten years the senior author has been developing a department of pharmacology to which the name of *phytopharmacology* has been applied. In this new field a comparative study was made in regard to the effects of various drugs, poisons, and toxins on living animal preparations, on the one hand, and living plant tissues, or plant protoplasm, on the other. These studies have led to some very interesting conclusions. It was discovered that living plant protoplasm may be much more sensitive to certain poisons than living animal protoplasm; and this was found to be particularly true of poisons or toxins elaborated by animals. By the use of quantitative phytopharmacologic methods, the first experimental evidence of the presence of various toxic substances in body tissues in certain pathologic conditions was obtained. Macht and Lubin showed the presence of a menotoxin or toxic substance in the blood, saliva, milk, perspiration and other secretions of women at the time of catamenia;¹ and these findings have since been confirmed by other investigators.^{2, 3, 4} Again, for the first time, Macht and Pels have demonstrated experimentally the presence of a toxic body in the blood serum and bullae of the baffling skin disease, pemphigus.⁵ Perhaps the most interesting findings so far obtained by Macht and his collaborators have been those resulting from their studies on pernicious anemia, the first of which was published in 1926.⁶ While making a routine examination of specimens of normal and pathologic blood sera from numerous patients, the senior author found that serum from pernicious anemia cases exhibited very marked and characteristic toxicity for living seedlings of *Lupinus albus* grown in

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cases makes the test of little value. The error of 13.1 per cent in the non-pregnant group makes the test valueless in the diagnosis of pregnancy."

The 519 tests reported here were done somewhat differently than originally proposed by Voge. Two tubes, in place of one, each containing 2½ c.c. of clear or filtered urine were used and to one 0.25 c.c. of bromine water was added, and to the other 0.5 c.c. of bromine water.* The test with the smaller amount of bromine was performed, since it was noted that in some pregnancy urines of low specific gravity (1.015 and less) the test with 0.5 c.c. of bromine was negative whereas with less bromine a definite pink color developed.

An analysis of the results obtained with the test using 0.25 c.c. of bromine water showed that while it increased the percentage of positive results in pregnancy urine from 54 per cent to 63 per cent it also increased the percentage of false positive results in nonpregnancy urine from 12 per cent to 16 per cent.

The quantitative feature of the test is explained by Hunter⁵ in a note on his modification of the Knoop test for histamine as follows: "The color produced is very soluble in an excess of bromine and in a dilute solution of histamine an initial excess may prevent all color development. The difficulty of adding just sufficient bromine water to slightly colored solutions has been overcome by adding a definite excess and washing out the excess with chloroform."

The simpler expedient of using two quantities of bromine also satisfies the quantitative requirements of the test.

A number of the positive results in our series were obtained in specimens from children. By deducting these tests and recalculating the percentage of false positive results, the total is 13 per cent which is similar to that obtained by Dodds whose specimens were all from women.

COMMENT

The Aschheim-Zondek and Friedman tests of urine for pregnancy are well known and their great accuracy and value have been well established by numerous workers. These biologic tests, however, requiring the use of mice or rabbits are expensive and time-consuming, and there is need for a simple chemical test for pregnancy. As reported above the Voge test is unsatisfactory for this purpose.

Von Mertz⁶ in 1928 proposed a chemical test for pregnancy on blood serum using as a test reagent phosphotungstic acid ($\text{H}_3\text{PO}_4 + 12\text{WO}_3 + 12\text{H}_2\text{O}$). He obtained 94.9 per cent agreement in 650 cases. Unfortunately this work has not been sufficiently confirmed and Kilduffe and Steinig⁷ have recently (1932) reported unsatisfactory results with it. They obtained a very low percentage of positive results (10.4 per cent) in pregnancy and approximately an equal number of positive results with nonpregnancy cases.

It still remains a problem to detect by a simple chemical means some substance in the urine or blood characteristic of the pregnancy state.

*The bromine water was prepared as follows: A stock solution of bromine water was made by placing in a glass stoppered bottle 6 c.c. of bromine (under a hood) and 300 c.c. of distilled water. After shaking for about forty-five minutes, a few cubic centimeters of the bromine remained undissolved and the supernatant solution was decanted. The stock solution therefore was about 1 to 1½ per cent of bromine. The stock solution diluted further with two parts of tap water constituted the test bromine water. The test bromine water was found to lose strength quite rapidly and was not used for more than one week after its preparation.

a dark incubator and kept at a temperature of 21° C. The increment in length of the seedlings obtained with stomach washings was then compared with the increase in length of the normal controls and expressed as the *phyto-toxic index*.

Complete details in regard to the technic of the phytopharmacologic test have been given in previous publications and need no repetition in this place. It may be well, however, to state emphatically that, for careful performance of the phytopharmacologic test, a great deal of training is required not only in ordinary zoöphysiologic and zoöpharmacologic methods but also in the laboratory methods pertaining to plant physiology and botany. A beginner expecting to succeed in such work on the first attempt will more than likely be disappointed. Indeed, he may not even be able to germinate the seeds, much less to secure uniform seedlings suitable for testing. The seeds employed in the present work were those of the large variety of *Lupinus albus*. These seeds must not be too old or worm-eaten and should be soaked for twenty-four hours in tap water to insure their complete swelling before being planted in the proper germinating medium. Finely ground sphagnum moss is the most suitable medium for germinating seeds. It is of vital importance, however, that such moss should be free from acid matter and contain just the proper amount of moisture, namely, 75 to 80 per cent its weight. The sphagnum moss should be thoroughly aerated and not closely packed; and, after planting, the seeds should be set aside in the dark and kept at a temperature no higher than 22° C. When a higher temperature is employed, growth may be so greatly stimulated as to mask small differences produced by inhibitory substances in the various solutions. For the performance of the test, the seedlings should have roots measuring from 30 to 40 mm. in length. Rows of eight, or ten seedlings should be employed for control solutions and for each of the solutions to be tested. To insure greater accuracy it is well to select seedlings of as nearly the same length as possible for corresponding tubes in each row.

RESULTS

The total number of cases studied was 125. The average phytotoxic index obtained from stomach washings of 100 patients, either normal or exhibiting various gastrointestinal disorders without pernicious anemia, was found to be 86 per cent. The lowest reading in these cases was 75 per cent. Table I gives a detailed analysis of the first 59 of these cases. Here are set forth the age, sex, and color of the patient, social status, the amount of free acid, the total acidity and Wassermann findings, the clinical diagnosis or impression of each case, the x-ray findings and, finally, the phytotoxic indices, obtained independently in the pharmacologic laboratory and with no previous knowledge of the history and physical findings of the various cases. It will be noted that of the 59 cases 11 gave phytotoxic indices ranging from 76 to 80 per cent; 31 gave phytotoxic indices between 80 and 90 per cent; and 15 cases gave phytotoxic indices between 90 and 100 per cent. Two cases gave a reading over 100 per cent, the highest being 101 per cent. The phytotoxic indices in these cases and of all the stomach washings which were

plant physiologic solution. The toxin was not menotoxin because it was present in male patients suffering from pernicious anemia, but it was not present in other blood diseases such as secondary anemia, carcinoma, leucemia, etc. The phytopharmacologic method therefore not only furnished a contribution to the etiology of Addison's or primary anemia but also offered a method of establishing a differential diagnosis between pernicious anemia and other anemias simulating it.⁷ Later investigations elaborating the phytopharmacologic method of study furthermore offered a convenient means of evaluating various methods of therapeutic procedure employed in the treatment of pernicious anemia.⁸ The findings obtained by means of this method of study in various diseases have been confirmed by numerous investigators, notably by Tschérkes,⁹ Tschérkes and Goldstein,¹⁰ Tschérkes and Mangubi,¹¹ Etinger and Artinov,¹² Stern,¹³ Herz and Weichbrodt,¹⁴ and Macht and Pels.¹⁵

More recently, Macht has demonstrated the presence of the pernicious anemia toxin not only in the blood but also in the spinal fluid of such patients.¹⁶ A comparative phytopharmacologic study of spinal fluids from various conditions had led to the discovery of a definite toxicity in the spinal fluid of patients suffering from true pernicious anemia. This method is already being employed by some physicians for differential diagnosis of neurologic signs and symptoms produced by pernicious anemia, on the one hand, and other conditions simulating it, on the other. These findings suggested the undertaking of the present investigation. It was deemed worth while to inquire whether the stomach washings obtained from various normal and pathologic conditions showed any difference in toxicity when examined by the new phytopharmacologic method.

METHOD

After various preliminary experiments the following method was adopted. The empty stomach of the patient was washed out with 100 c.c. of distilled water. This was done by introducing water through a clean stomach tube, which had been previously sterilized and washed free from any trace of antiseptic. The washings thus obtained were tested for their hydrogen ion concentration and then submitted for special examination to the pharmacologic laboratory. The phytopharmacologic method employed was the same as that used in the study of spinal fluid and blood.¹⁷ While a one per cent solution of the blood serum was employed in these experiments, a 2 per cent solution of the stomach washings was made because of the more dilute condition of the specimens at the outset. Such a 2 per cent solution was made in a plant physiologic saline consisting of equal parts of Shive solution and distilled water.¹⁸ Healthy young seedlings of *Lupinus albus* were placed in hard glass tubes containing such a liquid and the growth of their straight and well-defined single roots in normal physiologic saline was compared with the growth of roots of similar seedlings from the same crop and of approximately the same initial length, immersed in the physiologic solution of the stomach washing. The length of the roots was measured at the beginning of the experiment and twenty-four hours after the preparations had been placed in

TABLE I.—CONT'D

M.I.,	J26071	43	F	W	W	0	4	Negative	Achylia gastrica; chronic tonsillitis; chronic constipation; ventral hernia	Colon completely filled; no defects seen; lower half of colon markedly spastic	83%
W.B.,	J49850	27	M	W	M	16	24	?	Irritable colon	Trace of dye in gallbladder; nothing abnormal seen	76%
M.S.,	G14543	67	F	C	M	30	40	Negative	Hypertension; arteriosclerosis; functional dyspepsia; chronic constipation; hypertrophic arthritis	Partial obstruction in lower end of esophagus; impossible to make diagnosis of neoplasm	82%
E.V.,	J56343	26	F	W	M	38	56	Negative	Impression: psychoneurosis; constitutional inferiority; visceroposis	Barium enema; normal large intestine; colon completely filled; no defects seen	90%
W.M.,	H81618	55	M	W	M	28	42	Negative	Chronic cholecystitis	(Gallbladder does not fill with dye; very little dye in intestinal tract)	95%
M.L.,	G95080	40	F	W	M	30	40	Negative		Normal concentration of dye in shape, size and position normal; no stones seen; contracts and empties normally	77%
A.F.,	J48877	34	F	C	M	12	20		Chronic gastritis	Fluoroscope: stomach normal position, normal tone; no defect in either curvature; no intestinal barium	89%
G.G.,	J49849		(G. I. history lost)					Positive 4+			77%
S.L.,	J44410	30	M	W	S	46	60	Negative	Cholecystitis	Fluoroscope: colon, good position; stomach, steep-horn type	88%
A.F.,	J46052	48	M	W	M	36	40	Negative	Functional dyspepsia	Fluoroscope: stomach very high; pulled up to right irritable pylorus	77%
L.W.,	J25490	31	F	C	M	15	30	Negative	Splanchnoptosis with atony; upper right quadrant adhesions	Fluoroscope: adhesions right upper quadrant; ptosis; mild lower quadrant adhesions	82%
H.B.,	J46328	46	M	W	M	8	28	Negative	Chronic appendicitis	Fluoroscope: stomach moderately prolapsed to right and downward; moderate spasticity of pyloric region; hepatic flexure prolapsed; cecal stasis; transverse colon spastic and prolapsed	86%

TABLE I

NAME AND NUMBER	AGE	SEX	COLOR	SOCIAL STATUS	FREE ACIDITY	TOTAL ACIDITY	WASSER-MANN	DIAGNOSIS	X-RAY	PHYTO-TOXIC INDEX
M.W., H47411	54	F	W	M	4	28	Negative	Postoperative adhesions	Fluoroscope: colon spastic	85%
H.F., H57585	52	M	W	M	74	88	?	Impression: ulcer of duodenum (Paulson)	Spastic colon; no defects seen (B)	86%
E.H., H93408	35	M	W	S	18	28	Negative	Impression: cecal stasis	Nontubercular infiltration of both lungs	79%
H.D., J1204	35	M	W	S	16	34	Negative	Impression: possible ulcers; neurosis	Gallbladder series; no trace of dye in gallbladder, indicating some obstruction; no evidence of stones. Active hypermotile stomach; no defects in stomach or duodenum	90%
N.J., J24946	21	M	C	S	8	28	Positive 4+	Impression: reflex pyloro-spasm	Fluoroscope: some spasm at pyloric end and ptosis of transverse colon	89%
C.S., J40107	38	F	W	M	10	28	Negative	Psychoneurosis; hypertension; chronic constipation; functional dyspepsia; hypochlorhydria; aerophagia	Fluoroscope: moderate amount of cecal stasis; transverse colon prolapse slightly spastic	83%
T.H., J43492	27	M	C	M	6	24	Positive 4+	Functional dyspepsia	Fluoroscope; transverse colon, normal position; slightly spastic	76%
I.J., G10566	61	F	W	M	20	44	Negative		Fluoroscope: generalized viscerospasm; adhesions along colon; cyst form along clavicles; no dye enters gallbladder in series	90%
S.F., G98389	33	F	W	M	0	4	Negative	Impression: pelvic inflammatory disease; spastic colon	Fluoroscope: ptosis of colon; very atonic stomach; contour normal, cap normal	79%
R.G., G79393	41	F	W	M	10	26	Negative	Impression: cholecystitis	Gallbladder filled with dye and normal in outline; no stones seen (B)	99%
J.C., J48204	52	M	W		96 Histamine	100	?	(Some of history lost)	Fluoroscope: high spastic colon; second degree adhesions; right lower quadrant	76%
R.S., J59466	48	M	W	M	44	62	?	Postoperative adhesions; neurosis; postoperative hernia	Stomach normal in size, position and function; no lesion in stomach or duodenum	89%

TABLE I—Cont'd

M.W.	49	F	W	M	6	16	?	Cardiac hypertrophy; myocardial insufficiency; chronic constipation; menopause; postoperative adhesions	Fluoroscope: first degree splachnoplethosis and atony	87%
J.R., J6777	38	M	W	M	26	40	?	Impression: spastic constipation; dermatitis	Intestinal tract filled with dye; none in gallbladder	80%
W.K., J45128	40	M	W	M	28	56	Negative	No G. I. condition		94%
G.C., U19982	38	F	C	M	18	40	Negative	Ptois and atony; hyperchlorhydria	Fluoroscope: moderate degree of ptois	79%
E.H., J45666	50	F	W	S	0	10	Negative	Impression: organic obstruction of pylorus, possibly carcinoma; myocarditis; cardiac arrhythmia	Prolapsed hypertonic fish-hook stomach; no filling defect in stomach or duodenum	90%
D.S., J45499	23	F	C	M	18	42	Negative	Chronic appendicitis	Fluoroscope: lower right quadrant adhesions	88%
L.S., J46226	59	M	W	M	0	10	Negative	Achylia gastrica; gastrogenic diarrhea	Impossible to fill duodenal cap; no evidence of ulcer in stomach or duodenum; barium enema; normal large intestine	83%
M.B., H61540	42	M	W	S	64	82		Deformed?	Filling defect in duodenal cap due to ulcer	88%
L.L., H20529	41	F	W	M	38	60	?	Impression: psychoneurosis; functional dyspepsia	Fluoroscope: splachnoplethosis with atony	80%
L.S., H38052	43	M	W	M	12	22	?	Right upper quadrant adhesions; gastric neurosis	Very poor concentration of dye in gallbladder; normal in size; constacts and empties normally	89%
F.N., J58438	28	F	W	W	14	36	Negative	Spastic colon; spastic constipation; rectal fissures; psychoneurosis	Gallbladder filled with dye and normal in outline; no stones seen; good expulsion. Fluoroscope: fish-hook stomach; complete prolapse of transverse colon	90%
R.S., J4952	21	F	W	S	10	28	Negative	Functional dyspepsia; dysmenorrhea (antiflexion subacute)	Fluoroscope: ptois spastic colon	85%

TABLE I—CONT'D

NAME AND NUMBER	AGE	SEX	COLOR	SOCIAL STATUS	FREE ACIDITY	TOTAL ACIDITY	WASSER-MANN	DIAGNOSIS	X-RAY	PHYTO-TOXIC INDEX
C.T., J46326	30	M	W	M	28	52	?	Irritable colon and reflex pylorospasm	Fluoroscope: stomach in good position; fair tone; no filling defect in pylorus or duodenum; transverse colon in good position	95%
L.G., J45630	43	F	W	W	20	30	?	Postoperative adhesions	Fluoroscope: transverse colon spastic in true pelvis; evidence of cecal stasis; stomach, fish-hook type	80%
L.C., J37692	57	F	W	M	10	32	Negative	Hiccoughs; chronic constipation	Gallbladder filled with dye; no stones seen	88%
W.F., J44694	26	M	W	M	10	13	Negative	Visceroptosis	No dye in gallbladder; pyloric end of stomach constricted and irregular; duodenal cap not filled, suggesting ulcer	101%
C.S., J44018	35	M	W	M	60	66	?	Recurrent ulcer	Fluoroscope: bell-like, atonic stomach; cap, normal; large intestines, normal	91%
A.S., J43847	20	M	W	S	54	78	Negative	Chronic appendicitis	Very active stomach, occupying high position; no filling defect in stomach; duodenal cap slightly irregular; defect in duodenum; ulcer cannot be ruled out	95%
C.F., J43602	18	M	C	S	10	50	Negative	Functional dyspepsia; carious teeth; peptic ulcer (duodenal)	Large intestine, normal	78%
M.K., J43599	20	F	C	M	42	54	?	Impression: functional G. I. disorder with spastic G. I. tract		92%
J.K., J43128	52	M	W	M	0	4		Gastric carcinoma; achylia gastrica	Annular filling defect in pyloric end of stomach, suggesting neoplasm; pyloric end quite irregular; large filling defect on greater curvature, which may be due to pressure from mass	88%
E.D.	30	F	W	M	40	52	Negative	Obesity	Gallbladder filled with dye and normal in appearance	89%

studied bore no relation whatever to the hydrogen ion concentration of the solutions used because a 2 per cent solution of such gastric washings in plant physiologic saline, which normally gave a reading of P_H 4.8 to P_H 5.0, was too small to change the hydrogen ion concentration to a degree sufficient to affect the growth of seedlings. The hydrogen ion concentrations of the solutions were studied by both colorimetric and potentiometric methods (Table I).

The phytotoxic indices of the cases discussed above are of interest both in regard to positive and to negative findings. Thus, for instance, it was very interesting to find that the stomach washings from several cases of carcinoma ventriculi showed no difference in toxicity as compared with those of other normal or functional gastric conditions. The most important findings, however, were made in connection with the comparative study of a series of true or essential achylas, on the one hand, and the achylas of pernicious anemia, on the other. Table II exhibits the results obtained. A total of eight cases

TABLE II

ESSENTIAL ACHYLAS		ACHYLAS OF PERNICIOUS ANEMIA	
CASE	PHYTOTOXIC INDEX	CASE	PHYTOTOXIC INDEX
1 C - l	82%	1 B - t	60%
2 S - n	95%	2 A - s	52%
3 L - n	83%	3 F - s	47%
4 S - f	82%	4 C - s	63%
5 F - d	79%	5 C - e	55%
6 H - k	82%	6 A - n	67%
7 K - s	88%	7 B - r	63%
8 N - l	90%	8 S - r	63%
		9 S - y	62%
Average	85%	Average	59%

of essential or true achylia was studied. These cases were investigated clinically and all proved to be of a severe type which did not respond with secretion of hydrochloric acid even after injections of histamine. In all these cases, morphologic and microscopic blood examination excluded pernicious anemia. It will be seen that the average phytotoxic reading given by the eight cases was 85 per cent, which was the same as the figure obtained in the series of 100 cases mentioned above. On the other hand, nine cases of pernicious anemia with complete achylia, studied by the authors, yielded very definite and different results. These cases were diagnosed as pernicious anemia on the basis of complete clinical, physical, and laboratory tests as well as on the basis of phytopharmacologic examination of the blood specimens. The average phytotoxic index given by the stomach washings from these nine cases was 59 per cent, a figure far below that obtained in other gastrointestinal conditions (Table II).

DISCUSSION

The findings described above in connection with the study of different samples of stomach washings from various clinical conditions are obviously of manifold interest to the physician. In the first place, the toxic reaction of the specimens obtained from cases of primary anemia promises to be of value

TABLE I—CONT'D

NAME AND NUMBER	AGE	SEX	COLOR	SOCIAL STATUS	FREE ACIDITY	TOTAL ACIDITY	WASSER-MANN	DIAGNOSIS	X-RAY	PHYTO-TOXIC INDEX
M.K., U17993	35	F	W	S	0	4	Negative	Impression: no organic disease of G. I. tract	Fluoroscope: tremendously ptosed transverse colon; marked tenderness over entire abdomen	82%
M.G., U32693	39	M	C	M	0	10	Negative	Gastric ulcer	Defect in pyloric duodenal region, while not definite, sometimes suggests ulcer	85%
E.G., U13487	28	F	C	M			Negative	Exophthalmic goiter	No G. I. history to be found	88%
J.S., U33930	20	M	C	S	20	35	?	Impression: gastric ulcer	Faint concentration of dye in gallbladder; nothing abnormal seen	80%
J.P., G69165	68	W	W		22	32	?	Impression: constipation; old gastritis (alcoholic)	Fluoroscope: pylorus moderately hypernervetic; duodenum visualized and showed no defect	90%
S.P., G65869	27	F	C	S	4	16	Negative	Obesity; oral sepsis; functional dyspepsia	Large hypertonic stomach lying in transverse position; very active and irritable; no filling defect in stomach or duodenum	100%
A.H., J35949	64	F	W	W	0	10	Negative	Achlorhydria; gallbladder disease	No evidence of dye in gallbladder; no stones seen. Failure of organ to concentrate dye indicates gallbladder disease; definite hypertrophic arthritis of lumbar vertebrae	82%
W.H., H38314	33	M	C	M	0	6	Negative	Functional dyspepsia	Fluoroscope: barium seen in transverse colon and splenic flexure; the latter is high and somewhat redundant. Stomach in good position; no irregularities in contour	76%
M.S., J44536	54	F	C	M	8	40	?	Functional dyspepsia; spastic digestive tract	Fluoroscope: spastic G. I. tract	100%
E.F., J44685	20	M	W	S	44	66	?	Irritable G. I. tract	Fluoroscope: splanchnoptosis with atony	78%
E.S., J27565	22	M	W	S	8	50	Negative	?	Negative	87%
W.C., J43068	27	M	C	S	6	28	Negative	Gastric ulcer; hypertension	Fluoroscope and x-ray work not done	94%
F.B., U26585	43	F	W	S	24	34	Positive	Impression: syphilitic infection	?	84%

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in the differential diagnosis of the achylia characteristic of that disease from other forms of achylia. In the second place, the peculiar toxic reaction produced by stomach washings from pernicious anemia cases is a property to be borne in mind in theoretical discussions in regard to the etiology of that disease. In the third place, the present findings have an added interest in connection with the treatment of pernicious anemia since the relative toxicity of stomach washings from a given patient, before and after instituting any special method of therapy, will furnish an index as to its efficiency.

Problems of gastric acidity have been brought into renewed prominence by the current intensive studies of pernicious anemia and of cancer of the stomach. As is well known, both these diseases are characterized by anacidity or achylia. Moreover, Pollard and Bloomfield¹⁹ have recently called attention to the surprising number of persons presenting an inexplicable anacidity of the stomach. Consideration of the different types of achylia or anacidity should make any new method of differentiating between the various forms especially welcome. Thus, the recent introduction of histamine as a potent stimulus to the secretion of free acid by the gastric mucosa has been of considerable aid in classifying various forms of anacidity. The phytotoxic test promises to be another such aid because phytopharmacologic examination of stomach washings indicates a distinct and marked difference between essential achylia, resistant to histamine injections, and that of pernicious anemia. What light these findings may shed on the etiology of primary anemia we cannot say at present but the experimental data are none the less valuable. In this connection it may be well also to bear in mind the more or less successful results obtained in the treatment of pernicious anemia with desiccated stomach mucosa. It is hoped that the present report will stimulate further investigation along phytopharmacologic lines.

SUMMARY

1. A phytopharmacologic examination, according to a standardized technique, was made of stomach washings obtained from 125 clinical cases, who had received no food for at least twelve hours.

2. The average phytotoxic index of one hundred patients not suffering from achylia was found to be 6 per cent.

3. A few cases of gastric carcinoma showed no difference in toxicity from other clinical conditions of a benign or functional character.

4. The average phytotoxic index for nine cases of definite pernicious anemia with no free hydrochloric acid was 59 per cent.

5. The average phytotoxic index of eight cases of essential achylia, which failed to respond with secretion to injections of histamine, was found to be 85 per cent.

6. The difference in phytotoxic effects between stomach washings obtained from cases of pernicious anemia, on the one hand, and essential achylia, on the other, promises to be of clinical value in differentiation of the two conditions and warrants further studies along the lines described above.

The spectroscopic studies of the blood are of interest and significance, and are here given in detail. All blood samples were obtained from the median basilic vein. The first one, taken before treatment, had a distinct chocolate color. One part of this sample was diluted with 49 parts of distilled water, resulting in a reddish brown solution. This was examined spectroscopically, and showed the characteristic absorption bands of neutral methemoglobin between the C and D lines. A sample of blood taken twenty-four hours later, diluted as above, and examined spectroscopically, did not show any evidence of methemoglobinemia, but gave the characteristic absorption bands of oxyhemoglobin. (Plate I.)

Since the patient had been handling two distinctly different anilin products, parabromanilin, and parabromorthosulphanilic acid, animal experiments were undertaken to determine which of the two, or whether both of the substances were responsible for the poisoning of the patient.

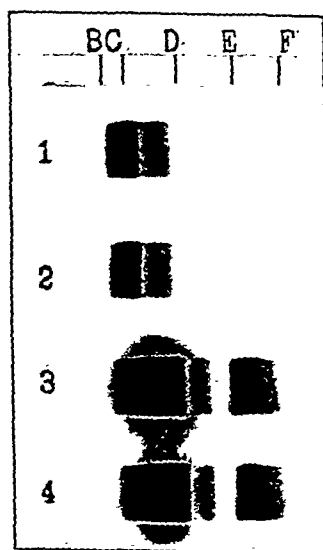


Plate I.—Showing spectrograms of blood of patient poisoned by contact with an anilin dye before and after the intravenous injection of methylene blue, as follows:

1. Blood of patient immediately before injection of methylene blue, showing pronounced neutral methemoglobin band.
2. Methemoglobin control.
3. Patient's blood one hour after injection of methylene blue showing normal oxyhemoglobin band.
4. Oxyhemoglobin of normal blood. Control.

Three rabbits were used. Rabbit No. 1 was a control, and received an injection of the 1 per cent methylene blue. Rabbit No. 2 was treated with an application of parabromanilin, in the form of a paste which was rubbed thoroughly into the skin. Rabbit No. 3 was treated in a similar manner with parabromorthosulphanilic acid. Rabbits No. 2 and 3 were later given injections of methylene blue.

Animal No. 2, treated with parabromanilin, became profoundly ill. Spectroscopic examination of the blood showed a marked neutral methemoglobinemia, which cleared up very rapidly after the injection of methylene blue.

Animal No. 3 showed no evidence of poisoning from the application of parabromorthosulphanilic acid, aside from a slight irritation at the site of

METHYLENE BLUE AS AN ANTIDOTE FOR ANILIN DYE POISONING^{*}

CASE REPORT WITH CONFIRMATORY EXPERIMENTAL STUDY

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THE recent press and technical journal notices regarding the use of methylene blue in the treatment of cyanide and carbon monoxide poisoning influenced the writers to try this procedure in a case of anilin dye poisoning, the details of which appear in the following report.

A male university student, aged twenty-three, while engaged in experimental work in a chemical laboratory, handled with his bare hands large quantities of para bromanilin and parabromorthosulphanilic acid. Intimate contact with the dye began about ten o'clock in the morning. In one portion of the experiment, while evaporating the material over an open flame, he inhaled the fumes for about an hour. At the end of this time it was noticed that his skin was becoming discolored. About twelve-thirty he left the laboratory and drove to his home. On the way he experienced a feeling of dizziness which gradually changed to severe headache. About 3 P.M. stupor developed. When seen at 4 P.M. the patient was semicomatose, had a severe headache, and the skin showed a discoloration suggesting an intense cyanosis. The color approximated a mauve lavender.

At this time the nature of the poisoning agent was not definitely known, and because of the grave condition of the patient, it was decided to remove him to the hospital and to try experimentally the methylene blue treatment as used by Geiger.

The patient was admitted to the Highland Hospital about 4 P.M. He was given at once, intravenously, 600 c.c. of 18 per cent glucose solution, plus 100 c.c. of 1 per cent methylene blue. Soon after the injection of this mixture was begun, the skin pigmentation began to disappear. In one hour the lips and nails became a normal pink color. The headache rapidly subsided. About 11 P.M. the patient was greatly improved and without headache. The skin, so far as could be observed, was normal in color.

Before instituting any treatment, and at irregular intervals thereafter, specimens of blood were taken for spectroscopic and other examinations. These disclosed that there was no disturbance in hemoglobin, red cell, white cell, or differential count. Blood sugar and blood nitrogen were also normal. The urine was normal both chemically and microscopically. A complete physical examination was made, which, except for the discoloration of the skin, was without significance. The pyrotannic test of the first sample of blood for carbon monoxide was normal.

^{*}From the Highland Hospital.

Rabbit No. 1. Male, weight 1725 grams, given 1 cubic centimeter of the methylene blue solution. Samples of blood were taken before, and at one hour and four hours after injection. Spectroscopic examination showed oxyhemoglobin bands, but no methemoglobin. (See Plate II, Spectra 1 and 2.)

Rabbit No. 2. Male, weight 1880 grams, parabromanilin paste poured on animal's back and rubbed in well with wooden spatula. At the end of 30 minutes the animal was in distress, having an accelerated heartbeat, and quickened breathing. At the end of the first hour, the rabbit was very ill, and showed evident collapse. A sample of blood taken and diluted with 49 parts of distilled water gave a reddish brown solution, which on spectroscopic examination showed a definite absorption band of methemoglobin. (See Plate II, Spectrum 3.)

At five hours the rabbit was much worse, weaker, unable to sit up in normal position, and leaning against the side of the cage for support. Breathing rapid. Marked cyanosis of

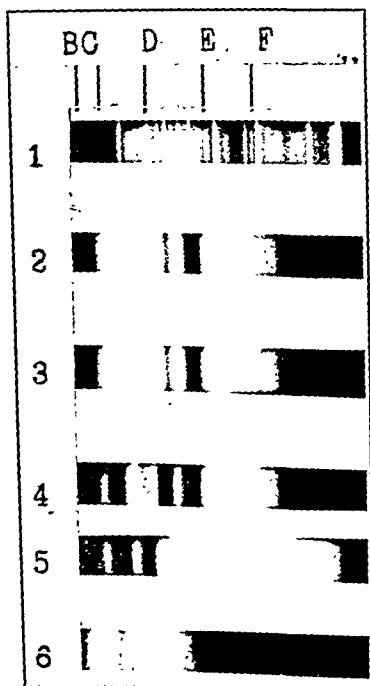


Plate III.—Showing spectrograms of control tests as follows:

1. Mercury arc.
2. Rabbit's blood before injection of methylene blue, normal oxyhemoglobin.
3. Rabbit's blood one hour after injection of methylene blue in twice the proportionate amount given to the patient, showing normal oxyhemoglobin.
4. Test tube mixture of normal blood and methylene blue. This seems to be a composite of the bands of oxyhemoglobin and methylene blue.
5. Dilute watery solution methylene blue.
6. Methemoglobin control.

ears. Second sample of blood taken, diluted as before, and examined spectroscopically. Blood chocolate color. Watery solution brown. Neutral methemoglobin bands very marked.

At six hours, rabbit very ill, and with no apparent improvement. Methemoglobin bands very strong. Rabbit given 1 cubic centimeter of methylene blue intravenously. (See Plate II, Spectrum 4.)

At seven hours, rabbit much improved, almost normal. Blood taken as before, and diluted with water. Blood normal in color, gives bright red solution. Spectroscopic examination shows normal oxyhemoglobin bands, no methemoglobin. (See Plate II, Spectrum 5.)

During this entire experiment the parabromanilin paste was not removed from the animal's back except as the rabbit itself licked and rubbed it away. After twenty-four

application. Spectroscopic examination of the blood showed the absorption bands of oxyhemoglobin, but no methemoglobin. (Plate II.)

The materials and apparatus used for this test, and details of the experiment are as follows:

1. Parabromanilin, 5 grams. Fifty per cent solution of ethyl alcohol and distilled water sufficient to make a thin paste.
2. Parabromorthosulphanilic acid, 5 grams. Fifty per cent solution of ethyl alcohol and distilled water sufficient to make a thin paste.
3. Methylene blue (Merck) medicinal, 1 per cent watery solution.
4. A Bausch and Lomb Universal Spectrophotometer, with camera attachment.

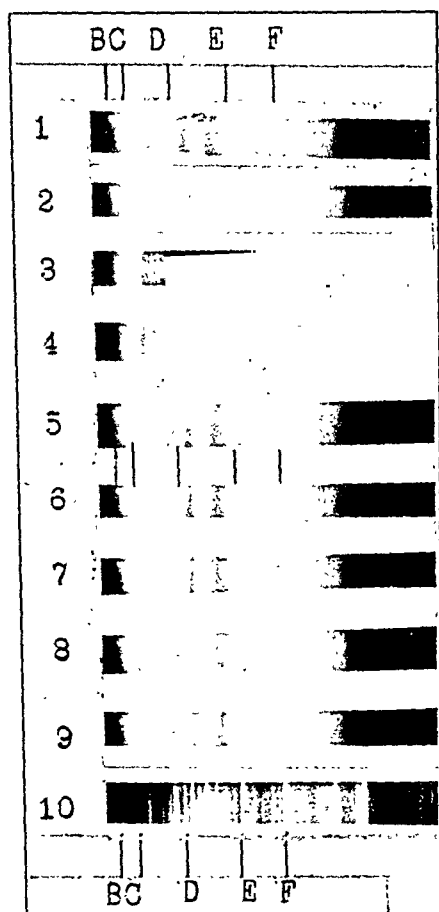


Plate II.—Showing spectrograms of bloods removed from experimental animals as follows:

1. Blood of Rabbit No. 1 before injection of methylene blue, showing the bands of oxyhemoglobin.
2. Blood of Rabbit No. 1 one hour after the injection of methylene blue, the oxyhemoglobin band being unaltered.
3. Blood of Rabbit No. 2 one hour after the application of parabromanilin to the skin, showing neutral methemoglobin.
4. Blood of Rabbit No. 2 at six hours, still showing a pronounced methemoglobin band.
5. Blood of Rabbit No. 2 one hour after injection of methylene blue. Methemoglobin band has been replaced by that of oxyhemoglobin.
6. Blood of Rabbit No. 2 after twenty-four hours, still showing oxyhemoglobin.
7. Blood of Rabbit No. 3 one hour after cutaneous application of parabromorthosulphanilic acid. Oxyhemoglobin present.
- 8 and 9. Same blood four and five hours later. Blood apparently not affected by the treatment.
10. Mercury arc.

Sahlin's work in 1930 and 1931, as cited by Hanzlik.² Geiger was apparently the first to make clinical application of the procedure. For a statement as to the history and priority of the method, the reader is referred to Hanzlik's communication.

Brooks³ found that methylene blue shortened the time of recovery from hydrocyanic acid poisoning to about one-third, and that from carbon monoxide to about one-half that of untreated animals. The results suggest that there is an activation of oxygen by methylene blue which manifests itself when aerobic oxidation is interfered with, and that it could be used advantageously in cases of hydrocyanic acid or carbon monoxide poisoning.

Geiger⁴ reports three cases of cyanide poisoning ending in death, where the usual restorative methods failed. In a fourth and similar case, the patient was saved by the intravenous use of methylene blue, the treatment being suggested in this case by Doctors P. J. Hanzlik and C. D. Leake, of the University of California Medical School. Geiger later reports two cases of carbon monoxide poisoning treated with favorable results by the use of methylene blue.

A fourth report is by Nass,⁵ who employed methylene blue in conjunction with carbon dioxide, oxygen inhalations, and other remedies in a case of illuminating gas poisoning, with successful outcome.

Since the preparation of this manuscript several communications have appeared in the literature, reporting the successful use of methylene blue in carbon monoxide poisoning. Haggard and Greenberg⁶ sound a note of warning against the employment of methylene blue as an antidote. They assert that its use is fraught with danger, and support by chemical experiment Wendel's contention that methylene blue forms methemoglobin in the blood.

Our clinical observations on this point do not sustain Haggard and Greenberg's conclusion. This investigation throws no light on the mechanism of the methylene blue action in the blood. That in itself is an interesting problem which awaits solution.

NOTE: The writers wish to acknowledge with sincere thanks the assistance of the technical research division of the Bausch and Lomb Optical Company.

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hours a fourth sample of blood was taken, and proved to be normal both in appearance and spectroscopic examination. (See Plate II, Spectrum 6.)

Rabbit No. 3. Male, weight 1460 grams, parabromorthosulphanilic acid rubbed into animal's skin, and blood taken at one, four, and five hours after application. Rabbit remained normal, and spectroscopic examination of blood showed no evidence of methemoglobinemia. (See Plate II, Spectra 7, 8, and 9.)

Since it has been reported by Wendel¹ that methylene blue injected intravenously produces methemoglobinemia, a male rabbit weighing 1650 grams was given 3.5 cubic centimeters of 1 per cent watery solution of methylene blue. Before the injection was completed, the bluish discoloration of the sclerae of the eyes began to appear. After fifteen minutes the sclerae were definitely blue, as was also the mucous membrane lining of the eyelids and mouth. At the end of one hour 0.5 cubic centimeters of blood was withdrawn, and diluted with 49.5 cubic centimeters of distilled water. This was examined spectroscopically. There was a marked shortening of the red end of the spectrum, but no evidence of the methemoglobin band. (See Plate III, Spectrum 2.) Blood was withdrawn again at the end of four hours, and examined with similar results. (See Plate III, Spectrum 3.)

For purposes of comparison, Plate III contains absorption spectra of oxyhemoglobin, neutral methemoglobin, and fresh blood mixed with methylene blue. (See Plate III, Spectra 1, 5, and 4.)

SUMMARY AND CONCLUSIONS

A case is described of anilin dye poisoning in a human being who had been handling parabromanilin and parabromorthosulphanilic acid, absorption presumably taking place from the skin. Intense signs and symptoms of pigmentation of the skin, headache, stupor, nausea, and vomiting rapidly supervened. These were promptly relieved by the intravenous injection of 1 per cent solution of methylene blue. Chemical, microscopic, colorimetric, and spectroscopic examinations were made of the bloods of both patient and experimental animals to determine the effect of cutaneous applications of the materials in question. It was definitely shown by spectroscopic studies of the blood that the poisoning in this case was due to parabromanilin, and that the parabromorthosulphanilic acid was nontoxic when applied to the skin.

The animal experiments indicated that methylene blue injected intravenously into a rabbit in approximately twice the strength of that given to the patient did not affect the absorption spectrum of normal blood, but if methylene blue be mixed with normal blood in vitro, the spectrum from such a mixture seems to be a composite of the spectra of methylene blue and normal blood. (See Plate III, Spectrum 4.)

A search of the literature fails to reveal a case of anilin dye poisoning resulting in methemoglobinemia in which the methemoglobin spectrum was changed to that of oxyhemoglobin with prompt clearing up of toxic signs and symptoms following the injection of methylene blue.

A brief review of the literature as to the use of methylene blue as an antidote is pertinent. The first to employ it thus was Sahlin of Lund, in 1926, who administered it in a case of cyanide poisoning, Eddy confirming

blood cells per c.mm. Fig. 1 shows that 94 per cent of the cases ranged between 4 and 5.6 million red blood cells per c.mm., while 6 per cent of the cases were outside of this range. Thirty-one boys had counts of more than 5 million red blood cells per c.mm., and 4 had counts less than 4 million red blood cells per c.mm. The lowest count was 3.28 million red blood cells per c.mm., and the highest count was 5.87 million red blood cells per c.mm.

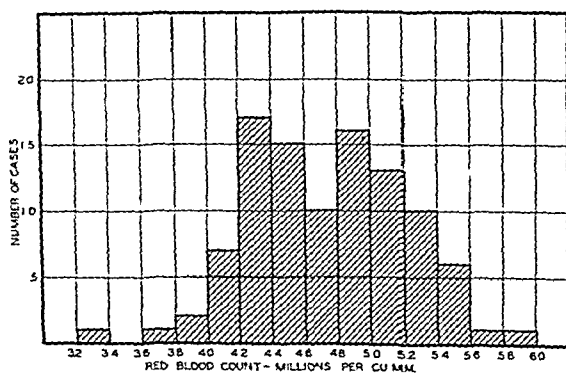


Fig. 1.—Distribution of red blood cell count in 100 boys between ages of twelve and seventeen years.

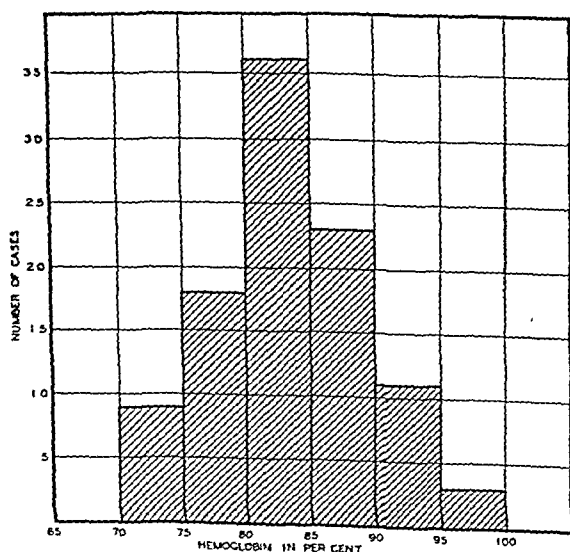


Fig. 2.—Percentage distribution of hemoglobin of 100 boys between the ages of twelve and seventeen years.

HEMOGLOBIN CONTENT

The inaccurate methods for the estimation of the hemoglobin content cannot be stressed too often. In a recent article¹⁰ the fallacy of individual estimations was also pointed out. Any hemoglobinometer may be employed as long as it is calibrated in grams of hemoglobin per 100 c.c. of blood. From this scale the percentage content of hemoglobin can be easily computed. Inasmuch

A STUDY OF THE RED BLOOD CELL COUNT AND HEMOGLOBIN IN THE ADOLESCENT MALE*

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THE necessity for establishing the normal red blood cell count and hemoglobin standard of the peripheral blood for the various age groups has become evident with the growing appreciation of the influence of various factors on the cell concentration in the circulating blood. Many of the standards which had been accepted were made without the proper critical data as to their source or accuracy. Accurate observations by Haden,¹ Rud,² Gram and Norgaard,³ Bing,⁴ Williamson,⁵ Komocki,⁶ Osgood,⁷ Wintrobe⁸ and others have been published, based on blood determinations carried out with painstaking technic in large series of individuals. As a result of their investigations, normals have been established for the various adult age groups. To these we wish to add the results obtained in a series of 100 boys in the adolescent stage.

The subjects of our study were healthy male students between the ages of twelve and seventeen years. From the data collected from various other studies, we have no reason to assume that any organic disease was present. All subjects were American born and the majority resided in the Great Lakes region all their lives.

The examinations were done in the fall of the year and throughout the entire day so that the averages eliminate diurnal variations.

The specimens of blood were obtained from punctures of the lobe of the ear. The first drop was discarded, the succeeding drops flowed freely so that no squeezing was necessary. The red blood cell counts were made on a Neubauer-Levy counting chamber (new ruling, U. S. Bureau of Standards). The pipettes used were checked by the U. S. Bureau of Standards. The hemoglobin was calculated in a Sahli hemoglobinometer in the usual manner with N/10 hydrochloric acid for one minute, diluted with distilled water, and compared with a colored rod standard. In compiling the results to be discussed, the Sahli hemoglobinometer in which 14 gm. of hemoglobin were equivalent to 100 per cent, was used because of its convenience. The instruments were calibrated and checked in accordance with the Van Slyke gas method⁹ for the estimation of hemoglobin.

RED BLOOD CELL COUNTS

As far as can be determined no large series of cases have been reported concerning the average red cell count in the adolescent stage. Our average in 100 boys between the ages of twelve and seventeen years was 4.71 million red

*From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan.

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TABLE I--CONT'D

SUBJECT	AGE	RED BLOOD CELLS MILLIONS PER C.MM.	HEMOGLOBIN		C. I.	HEMOGLOBIN COEFFICIENT	
			%	GM.		%	GM.
51	14	5.07	85	11.90	0.84	83.3	11.62
52	14	5.08	78	10.92	0.77	76.4	10.71
53	14	4.71	83	11.62	0.88	87.9	12.32
54	14	4.79	75	10.50	0.78	78.0	10.92
55	14	5.10	82	11.48	0.80	80.3	11.77
56	14	4.05	78	10.92	0.95	96.0	13.65
57	14	4.59	79	11.06	0.85	85.3	11.97
58	14	5.13	82	11.48	0.80	80.3	11.27
59	15	4.32	70	9.80	0.81	81.4	11.76
60	15	4.52	82	11.48	0.91	90.2	12.74
61	15	4.0	74	10.36	0.92	92.0	12.88
62	15	5.40	90	12.60	0.83	82.8	11.62
63	15	5.51	89	12.46	0.81	80.9	11.34
64	15	4.39	79	11.06	0.89	89.0	12.18
65	15	4.96	84	11.76	0.85	85.0	11.76
66	15	4.99	88	12.32	0.88	88.0	12.32
67	15	5.05	74	10.36	0.73	72.5	10.15
68	15	5.45	93	13.02	0.85	85.5	11.97
69	15	4.44	90	12.60	1.01	101.0	14.14
70	15	4.44	87	12.18	0.98	98.0	13.37
71	15	4.90	86	12.04	0.87	86.0	12.04
72	15	4.89	95	13.30	0.98	97.0	13.30
73	15	4.56	87	12.18	0.95	93.9	13.16
74	15	4.84	93	13.02	0.96	96.7	13.51
75	15	4.42	85	11.90	0.96	96.0	13.09
76	15	5.69	81	11.34	0.71	69.6	9.73
77	15	4.68	93	13.02	1.00	99.0	13.86
78	15	5.48	93	13.02	0.84	84.6	11.83
79	15	5.14	86	12.04	0.84	84.2	11.76
80	15	4.40	79	11.06	0.89	89.0	12.46
81	15	4.27	83	11.62	0.97	96.2	13.44
82	15	4.32	83	11.62	0.96	96.2	13.44
83	15	4.91	83	11.62	0.84	83.8	11.76
84	15	4.14	82	11.48	1.00	98.4	13.79
85	15	4.15	75	10.50	0.91	90.0	12.60
86	16	4.70	90	12.60	0.95	96.3	13.44
87	16	4.45	94	13.16	1.05	103.4	14.59
88	16	5.31	94	13.16	0.88	88.2	12.32
89	16	4.60	85	11.90	0.92	91.8	12.88
90	16	5.05	89	12.46	0.88	87.2	12.18
91	16	4.24	85	11.90	1.00	100.0	14.28
92	16	3.28	83	11.62	1.27	126.0	17.43
93	16	4.67	84	11.76	0.88	89.0	12.46
94	16	5.19	92	12.88	0.88	89.3	12.53
95	16	4.63	93	13.02	1.01	100.4	14.07
96	16	5.21	97	13.58	0.93	93.1	13.02
97	16	4.87	78	10.92	0.80	79.0	11.06
98	16	4.45	88	12.32	0.99	99.0	13.86
99	17	4.38	79	11.06	0.89	90.0	12.60
100	17	4.34	88	12.32	1.02	102.0	14.28
Average		4.718	82.57	11.55	0.87	88.26	12.35

TABLE I
OBSERVATIONS ON THE BLOOD IN ONE HUNDRED ADOLESCENT MALES

SUBJECT	AGE	RED BLOOD CELLS MILLIONS PER C.M.M.	HEMOGLOBIN		C. I.	HEMOGLOBIN COEFFICIENT	
			%	GM.		%	GM.
1	12	4.44	82	11.48	0.92	91.0	12.74
2	12	4.38	75	10.5	0.85	82.5	11.55
3	12	4.82	71	9.94	0.74	73.8	10.36
4	12	4.28	85	11.90	1.00	98.6	13.80
5	12	5.41	75	10.50	0.70	69.0	9.66
6	12	4.50	81	11.34	0.90	89.1	12.46
7	12	4.18	81	11.34	0.97	97.2	13.58
8	12	5.38	80	11.20	0.75	73.6	10.29
9	13	4.53	75	10.50	0.83	82.5	11.62
10	13	5.23	80	11.20	0.77	76.8	10.28
11	13	4.55	70	9.80	0.78	77.0	10.78
12	13	4.01	81	11.34	1.00	99.2	13.86
13	13	4.82	82	11.48	0.85	86.0	12.04
14	13	4.62	85	11.90	0.92	91.8	12.88
15	13	5.06	83	11.62	0.82	82.0	11.48
16	13	5.55	83	11.62	0.75	75.5	10.57
17	13	5.04	76	10.64	0.75	74.4	10.36
18	13	4.40	83	11.62	0.94	94.3	13.16
19	13	4.76	83	11.62	0.87	87.9	12.32
20	13	4.86	85	11.90	0.87	85.0	11.90
21	13	4.84	84	11.76	0.87	87.3	12.18
22	13	4.36	83	11.62	0.95	96.2	13.44
23	13	5.87	70	9.80	0.60	60.2	8.40
24	13	4.80	85	11.90	0.88	88.4	12.39
25	13	5.20	80	11.20	0.77	76.8	10.78
26	13	5.20	85	11.90	0.81	81.6	11.41
27	13	4.37	74	10.36	0.85	85.0	11.48
28	13	4.63	83	11.62	0.90	89.6	12.53
29	13	4.80	80	11.20	0.83	83.2	11.62
30	13	4.04	81	11.34	1.00	101.0	14.14
31	13	4.80	76	10.64	0.79	79.0	11.06
32	13	3.84	70	9.80	0.92	91.7	12.91
33	13	3.90	83	11.62	1.03	103.7	14.59
34	13	4.92	81	11.34	0.82	81.0	11.34
35	13	5.05	82	11.62	0.81	81.0	11.34
36	13	3.73	81	11.34	1.08	108.0	15.12
37	13	4.28	71	9.94	0.83	82.3	11.55
38	13	4.45	78	10.92	0.87	87.0	12.18
39	13	5.30	78	10.92	0.73	74.8	10.50
40	13	4.33	82	11.48	0.95	95.1	13.30
41	13	5.28	89	12.46	0.85	83.6	11.69
42	14	4.24	97	13.58	1.14	114.0	16.31
43	14	5.06	85	11.90	0.84	85.0	11.90
44	14	4.94	87	12.18	0.88	87.8	12.32
45	14	5.21	87	12.18	0.83	83.5	11.69
46	14	4.31	81	11.34	0.94	93.9	13.16
47	14	4.24	75	10.50	0.89	90.0	12.60
48	14	5.10	88	12.32	0.86	86.2	12.04
49	14	4.22	82	11.48	0.97	98.4	13.72
50	14	5.22	78	10.92	0.75	74.8	10.50

2. The average hemoglobin estimation is 11.55 gm. per 100 c.c. of blood or 82.57 per cent, the range being 9.80 gm. (70 per cent) and 13.58 gm. (97 per cent) per 100 c.c. of blood.

3. The average hemoglobin coefficient for the 100 cases is 12.35 gm. per 100 c.c. of blood.

4. The average color index for adolescent males is 0.87, the lowest index is 0.60 and the highest color index is 1.27.

5. If the hemoglobin coefficient be expressed in percentage rather than grams, it assumes approximately the same figure as the color index.

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as the hemoglobin varies with sex, age, time of day, altitude, climate, and race, the necessity for definite standards for the various age groups is indicated.

In the 100 cases herein reported, the average hemoglobin estimation was 11.55 gm. per 100 c.c. of blood or 82.5 per cent (Sahli). The lowest value was 9.80 gm. or 70 per cent (Sahli) and the highest value 13.58 grams or 97 per cent (Sahli). Of all the estimations, 88 per cent were between the range of 75 and 95 per cent (Sahli) or 10.50 to 13.30 gm.; 9 per cent of the cases were less, and 3 per cent of the cases were more than this (Fig. 2). The average hemoglobin content of each red cell was 26^{-12} gm., which is slightly less than the value for adult males as computed by Osgood.⁷

INDICES

The color index refers to the amount of hemoglobin in the average cell, expressing the amount in proportion to the normal. Table I contains the figures from which the various indices have been computed. The average uncorrected

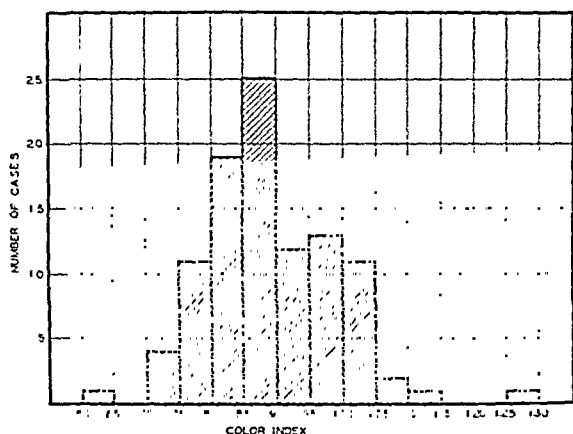


Fig. 3.—Color index distribution in 100 boys between the ages of twelve and seventeen years.

color index for the 100 cases is 0.87. The lowest index is 0.60 and the highest 1.27. In Fig. 3 it is noted that 83 per cent of the cases had a color index within the range of 0.80 to 1.15. Sixteen per cent of the cases had a lower color index and 1 per cent had a higher color index.

Curiously enough, if the hemoglobin coefficient be expressed as the percentage rather than the grams of hemoglobin per 5.0 million red blood cells, it has approximately the same figure as the color index. In computing the hemoglobin coefficient, it is noted that the average is 12.35 gm. per 100 c.c. which is a slightly higher value than the hemoglobin average. However, neither of these figures approaches the accepted standard for adults of 14 to 16 gm., which is recognized by the majority of investigators.

CONCLUSIONS

1. The average red blood cell count in males between the ages of twelve to seventeen years is 4.718 million per c.mm., the range being 3.28 to 5.87 million red blood cells per c.mm.

rate for lead had been increased by the lead injections, but the effects were irregular. Millet¹² was unable to find evidence that the administration of colloidal lead orthophosphate altered the rate of lead excretion in the urine of patients. Newman, on the other hand, found a definite response in the horse,⁸ and in patients,¹³ after intravenous administration of colloidal lead orthophosphate, although the increase in the urinary lead concentration was small, and slow to make its appearance, as compared to the results obtained when colloidal lead had been injected.^{8, 14}

We have studied the activity of certain insoluble particulate lead preparations in animals by methods similar to those previously employed in the study of soluble lead salts,¹⁵ and tetraethyl lead.¹⁶ We have made some observations on patients who have been treated for inoperable cancer with colloidal lead preparations. The animal experiments were designed to determine the character of the distribution of lead in the tissues, and the rate of its excretion not only immediately following injection, but also over subsequent periods during which the lead might be expected to migrate in the body.

EXPERIMENTAL METHODS

Five preparations were employed for intravenous administration.

1. A suspension of finely divided crystals of tertiary lead phosphate prepared by adding 1.5 per cent Na_3PO_4 to 0.5 per cent PbCl_2 . The mixture was filtered at once, the precipitate was thoroughly washed on the filter paper but not dried, resuspended in distilled water, and used immediately.
2. A suspension of crystals of the secondary lead phosphate prepared in similar fashion, by using the secondary sodium phosphate as the precipitant.
3. Colloidal lead orthophosphate stabilized with gelatin prepared by the method described by Bischoff and Blatherwick.¹¹
4. Colloidal lead orthophosphate procured from the Crookes Laboratories.
5. Colloidal lead from the same source.

The suspensions of crystalline phosphates were used to determine the influence of relatively large particles on the distribution of lead in animals, and to eliminate any uncertainty as to the chemical nature of the material injected. They were somewhat unsatisfactory as materials for intravenous injection, because of their tendency to block the syringe. Thus the amounts injected can only be estimated approximately. A small amount of blood was allowed to flow back into the syringe and after vigorous agitation of the contents of the syringe, the material was slowly injected. Death from embolism occurred in a few instances, but when the injection was carried out slowly the animals showed no ill-effects from any dosage employed. No attempt was made to determine the size of the particles of these suspensions.

Healthy full-grown rabbits were employed as experimental animals. Except for those that died as a result of the injection, they were sacrificed for analysis at suitable times, by bleeding from the heart under light ether anesthesia. Some of them were kept in metabolism cages from the time of injection until they were sacrificed, to follow their lead excretion. When this was done parallel observations were made on control animals. In other instances a control

THE BEHAVIOR OF LEAD IN THE ANIMAL ORGANISM*

III. COLLOIDAL LEAD COMPOUNDS

ROBERT A. KEHOE, M.D., AND FREDERICK THAMANN, CH.E., CINCINNATI, OHIO

INTRODUCTION

THE use of colloidal lead compounds in the treatment of malignant neoplasms has introduced a new factor into the problem of the physiologic behavior of lead. It may not be assumed that particulate lead compounds, injected intravenously, act in the same manner as those which are absorbed through the alimentary tract and the respiratory membranes. Thus, it is not surprising that some of the observations which have been made, following the administration of colloidal lead compounds, are not in harmony with previous experimental findings.

A review of the literature discloses certain facts upon which investigators are in agreement. Shortly after the injection of colloidal lead preparations the largest amounts of lead are found in the spleen and liver.^{1, 2, 3, 4, 5, 6, 7} Frequently the lungs contain large amounts of lead. At this time the skeleton shows relatively low concentrations, while the kidneys, heart muscle, somatic muscle, brain, testicles and intestinal tract (without contents) show variable amounts. The blood stream loses its lead at a rapid rate after an injection. Bischoff⁴ and his associates have described experiments in which virtually all the lead given as tri-lead orthophosphate was removed from the blood in two hours. Newman's⁸ observations on horses showed that about 90 per cent of colloidal tri-lead orthophosphate disappeared from the blood in the first hour, while at the end of the second hour the blood was practically free of lead. In the case of colloidal lead, the removal from the blood stream was not so rapid, nor so complete, considerable amounts remaining in the blood sixty-six hours after the injection. Both chemical and histologic evidences point to the initial distribution of the colloidal compounds in the reticuloendothelial system,^{6, 9, 10} while according to Henriques and Okkels⁹ the administration of soluble salts promotes a contrasting deposition of lead in parenchymal cells of the liver and kidney.

The ultimate fate of the colloidal lead preparations in the tissues has not been studied adequately. Apparently, the importance of time as a factor in the dynamic processes of redistribution and excretion, has not been appreciated. Bischoff and Blatherwick¹¹ have mentioned fragmentary observations as evidence that injected lead orthophosphate induces an increased lead excretion. Todd³ reports the study of the lead excretion of a patient treated with lead selenide. One would conclude from his results that the excretory

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TABLE I

CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAPSE OF TIME FOLLOWING INTRAVENOUS ADMINISTRATION OF LEAD CHLORIDE SOLUTION

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Mg. of Lead Administered	6 mg. in 1 dose										15 mg. in 2 doses in 1 wk. apart										12 mg. in 2 doses in 1 wk. apart										12 mg. in 2 doses in 1 wk. apart										12 mg. in 2 doses in 1 wk. apart										12 mg. in 2 doses in 1 wk. apart																																																																					
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	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
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	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
Time Interval After Last Injection	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
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	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
Time Interval After Last Injection	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
Time Interval After Last Injection	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
Time Interval After Last Injection	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
Time Interval After Last Injection	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									
Time Interval After Last Injection	10 min.										30 min.										2 hr.										16 hr.										11 days										1 mo.										6 wk.										3 mo										5 mo.										7.5 mo.										9 mo.										11 mo.									

period of excretion preceded the lead injection. Other animals kept for longer periods of time after injection, were introduced into the metabolism cages for a short time just before they were sacrificed. In this way information as to the magnitude of lead excretion at long intervals after treatment was provided.

The care of the animals, the excretory studies, the preparation of animal tissues, and the analytical procedures were carried out in the manner described in previous articles.^{16, 17}

The observations on patients were made with the assistance of L. J. Schradin. The urine and feces were collected in containers provided by the laboratory. Precaution was taken against contamination of these samples from external sources. Tissues obtained at necropsy were washed in distilled water while fresh and stored in chemically clean jars.

RESULTS

We have given elsewhere the characteristic distribution of lead in the tissues of rabbits a long time after intravenous administration of lead salts.¹⁵ Examples have appeared in earlier articles, illustrating the changes in the distribution¹⁵ and excretion¹⁶ of lead, which take place with the lapse of time following the intravenous injection of soluble lead salts into rabbits. The more complete data given in this paper will permit detailed comparison of the behavior of soluble lead salts with that of colloidal compounds. Table I shows the results of the analyses of the tissues of twenty-four rabbits that had received lead chloride intravenously. These animals died or were killed after various periods of time following the lead injections. Certain tissues were not analyzed separately. Rather, the attempt was made to select those organs which gave the greatest promise of information as to the distribution of lead. The mixed tissues which, for one reason or another, were not separated into individual samples, were grouped as one marked "remainder," or were discarded. The skin, ears, and feet were usually discarded because of the possibility of their external contamination, the ears as the sites of original injection being particularly under suspicion. In the case of several animals that were employed for excretory studies, there was no such question with reference to the ears because of the time interval between injection and death. Furthermore, the skin of these animals had been carefully cleansed at the beginning of the excretory observations, and again at the death of the animal. Such animals were analyzed in their entirety.

To clarify matters, the data are expressed in three forms. The upper section of the table shows the actual quantities of lead found by analysis; the middle section gives the calculated concentration in milligrams per one hundred grams of the tissue; the lower section relates the concentration in any one tissue to the concentration in the combined mass of tissue analyzed. This demonstrates the selective nature of the localization, and allows the analytical results in one animal to be compared with those in another regardless of the absolute amounts of lead present in the tissues.

Within a few minutes to two hours after intravenous injection of lead chloride, practically all the tissues examined showed the presence of lead. Un-

TABLE I
CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAISE OF TIME FOLLOWING INTRAVENOUS ADMINISTRATION OF LEAD CHLORIDE SOLUTION

TREATMENT OF LEAD CHLORIDE POISONING																								
IDENTIFICATION NUMBER	243	248	256	252	238	237	257	212	256	251	261	244	250	253	255	206	171	172	254	203	205	176	249	278
Mg. of Lead Administered	6 mg. in 1 dose						28 mg. in 1 dose	12 mg. in 2 doses of 6 mg. each 1 wk. apart						1 mg. and 6 mg. wk. apart		6 mg. doses of 1 mg. wk. apart		15 mg. in 1 dose		26 mg. in 1 dose		12 mg. and 6 mg. 3 mo. apart	6 mg. in 1 dose	
Time Interval After Last Injection	10 min.		10 min.		2 hr.	16 hr.	14 days		1 mo.		6 wk.		3 mo.		5 mo.	7.5 mo.		9 mo.		11 mo.				
Lead in Milligrams Found in Tissues																								
Kidneys	1.41	0.19	trace	0.17	0.50	0.07	0.19	0.00	trace	0.00	0.08	0.09	0.00	trace	0.00	0.35	0.03	0.01	0.00	0.05	0.20	0.08	0.04	0.03
Liver	1.86	0.66	1.50	0.40	2.45	1.00	1.20	0.25	0.20	0.10	trace	0.21	0.09	0.00	0.10	0.37	0.19	0.08	trace	0.13	0.03	0.00	0.15	0.05
Spleen	0.00	0.00	0.60	trace	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	trace	0.00	0.03	0.01	0.00	0.04	0.05
Fat	0.33	0.00	0.03	0.10	0.23	0.00	0.00	0.00	0.24	0.00	0.03	0.00	0.00	0.05	0.00	0.33	trace	0.06	0.07	0.18	0.05	0.05	0.06	0.21
Central nervous system	0.03	0.00	0.05	0.00	0.74	trace	0.00	0.13	trace	0.00	0.00	0.07	0.00	0.00	0.00	0.09	0.00	0.11	0.00	0.12	0.03	0.03	0.03	0.00
Blood	0.12	0.10	0.09	0.07	1.59	trace	0.12	0.17	0.08	0.00	0.06	0.00	0.07	0.03	0.03	0.03	0.03	0.03	0.06	0.01	0.06	0.00	0.00	0.05
Muscle	0.61	0.30	0.75	0.15	2.50	0.28	0.36	0.35	0.16	0.27	0.14	0.11	0.00	0.12	0.23	0.35	0.35	1.05	0.81	0.21	0.05	0.05	0.01	0.11
Bone	0.95	1.05	1.25	2.70	1.76	1.40	0.98	3.35	2.60	0.77	2.15	3.13	1.98	2.20	2.00	4.21	1.11	1.05	1.08	1.71	0.37	0.71	0.69	0.07
Washed int. tract	0.42	0.51	0.51	0.18	1.77	0.57	0.10	0.11	0.00	0.05	0.06	0.00	0.00	trace	0.00	0.01	0.01	0.12	0.03	0.06	0.03	0.16	0.54	0.54
Skin							0.60	0.71			1.46	1.16	0.36	0.00	0.25	0.48	0.10	0.10	0.06	0.01	0.01	1.12	2.27	2.27
Remainder	5.73*	3.01*	5.11*	1.79*	11.96*	1.32*	2.05*	1.70	3.28*	1.10*	2.82*	5.10	3.66	2.40*	3.61	5.81	1.72*	1.65*	1.70	2.53*	0.81*	0.93*	2.33*	3.33*
All tissues																								
Lead in Milligrams Per 100 Grams Tissue																								
Kidneys	10.33	1.19		0.81	2.17	0.37	1.00	0.00	0.00	0.41	0.60	0.00	0.00	0.00	0.00	2.92	0.18	0.29	0.00	0.38	1.82	0.80	0.17	0.17
Liver	2.14	0.17	1.69	0.37	1.99	1.06	1.29	0.25	0.27	0.18	0.25	0.12	0.00	0.11	0.44	0.44	0.21	0.09	0.00	0.11	0.06	0.00	0.12	0.05
Spleen	0.00	0.00	20.00	0.67	9.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.00	0.00	0.06	0.13	1.00	0.00	1.60	0.00	0.00
Fat	1.72	0.00	0.05	0.35	0.21	0.00	0.00	0.00	0.21	0.00	0.09	0.00	0.00	0.19	0.00	0.91	0.00	0.06	0.00	0.03	0.01	0.01	0.01	0.04
Central nervous system	0.21	0.00	0.33	0.00	1.35	0.00	0.00	0.03	0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.69	0.00	0.79	0.00	0.21	0.00	0.14	0.00	0.00
Blood	1.33	2.50	1.00	0.70	8.37	1.41	1.19	0.00	0.11	0.00	0.05	0.00	0.08	0.01	0.03	0.07	0.00	0.01	0.08	0.01	0.09	0.00	0.01	0.01
Muscle	0.10	0.09	0.07	0.02	0.22	0.03	0.05	0.01	0.03	0.04	0.02	0.01	0.00	0.02	0.02	0.05	0.05	0.16	0.01	0.02	0.01	0.01	0.01	0.01
Bone	0.48	0.62	0.88	1.90	0.99	0.82	0.69	1.48	1.83	0.51	1.21	1.58	1.00	1.11	0.88	2.50	0.19	0.16	0.95	0.75	0.19	0.31	0.30	0.02
Washed int. tract	0.21	0.21	0.59	0.09	0.99	0.40	0.06	0.06	0.00	0.01	0.03	0.00	0.00	0.00	0.00	0.02	0.02	0.09	0.02	0.04	0.03	0.00	0.07	0.27
Skin							0.21	0.21			0.18	0.21	0.11	0.06	0.00	0.08	0.01	0.01	0.01	0.03	0.02	0.08	0.13	0.13
Remainder							0.17	0.17			0.88	1.35	0.79	0.46	0.21	0.02	0.01	0.01	0.10	0.02	0.02	0.08	0.13	0.13
Relative Concentration of Lead in Tissues																								
Mg. per 100 Grams of Tissue																								
Kidneys	20.75	1.43		2.95	3.22	1.61	1.02			2.23	2.88					7.33	1.51	2.86		4.00	31.40	2.60	2.60	2.30
Liver	1.28	1.76	5.12	1.36	2.95	4.71	5.18	0.96	0.97	1.68	1.22	0.67	0.85			1.21	1.80	0.93		1.10	1.13	1.81	0.65	0.65
Spleen			66.67	2.43	13.83											7.51				10.12	75.47	21.88	0.57	0.57
Fat	9.47		0.15	1.25	0.36				0.71	0.41		0.86				2.57		0.56	1.22	0.31	0.28	0.66		
Central nervous system	0.43		1.00	6.45			3.65				2.10					1.71		7.85		10.12	4.04	2.09	0.44	0.44
Blood	2.68	9.33	3.00	2.55	12.40	5.67	0.72	0.10	0.45	0.19	0.21	0.18	0.43	0.71	0.46	0.18	0.39	0.43	0.71	0.46	1.70	0.01	0.01	0.01
Muscle	0.20	0.35	0.22	0.07	0.33	0.12	0.20	0.17	0.09	0.06	0.06	0.10	0.18	0.10	0.18	0.05	0.18	0.05	0.05	0.18	0.11	0.11	0.04	0.04
Bone	0.96	2.31	2.67	6.91	1.47	3.66	2.77	5.78	6.63	5.22	7.58	5.59	5.11	6.83	6.28	1.18	1.63	8.77	7.81	7.28	4.54	0.29	0.29	0.29
Washed int. tract	0.43	0.89	1.79	0.33	1.47	1.78	0.21	0.22	0.31	0.15	0.15					0.01	0.21	0.01	0.02	0.36	0.19	1.04	3.54	3.54
in remainder							0.82				0.88	1.35	0.79	0.46	0.21	0.02	0.01	0.01	0.10	0.02	0.42	1.27	1.69	1.69

*Skin and/or mixed tissues ("Remainder") discarded.

doubtedly some of this lead was in the residual blood of these tissues, and since the blood was high in lead at this time, this factor is important. However, in some organs the concentration of lead was higher than would be expected from their blood content, while the reverse was true in others. In general the kidneys, liver, spleen, and the blood were especially high in their lead content, though there was a considerable variability in the relative positions of these solid organs. Muscle tissue was uniformly low in lead, and, with one exception, the bones. (The exception [Rabbit 252] is the result of the injection of 6 mg. of lead a week before the final injection.) The amounts in the bones are somewhat higher than would be expected immediately after injection. No doubt the vascular bone marrow was responsible for a certain quantity, but the additional factor of lead present before the experimental injection may not be ignored. We have found that the lead in normal, untreated rabbits varies in amounts from 0.29 mg. to 2.60 mg., as shown in Table II and Table III. The chief influence of such normal lead in these experiments is to magnify the lead content of the skeletal tissues.

The only significant difference in the distribution of lead sixteen hours after injection is seen in the result obtained on the blood of Rabbit 237, which showed only a trace of lead. The data of these experiments are not adequate to show the time at which the greater proportion of injected lead disappears

TABLE II
LEAD FOUND IN NORMAL RABBITS BY ANALYSIS OF ENTIRE CARCASS

IDENTIFICATION NUMBER	PB IN MG.	IDENTIFICATION NUMBER	PB IN MG.
133	0.00	93	1.40
111	0.29	C ₂	1.50
119	0.45	89	1.70
P ₁	1.10	98	1.70
C ₁	1.23	121	2.60

TABLE III
THE DISTRIBUTION OF LEAD IN UNTREATED (CONTROL) RABBITS

IDENTIFICATION NUMBER	364	373	361
<i>Lead in Milligrams Found in Tissues</i>			
Kidneys	0.00	0.00	0.00
Liver	0.00	0.00	0.00
Bile	0.00	0.00	0.00
Spleen	0.00	0.00	0.00
Fat	0.00	0.00	0.00
Central nervous system	0.00	0.00	0.00
Blood	0.00	0.00	0.00
Muscle	0.00	trace	0.00
Bone	0.00	0.04	0.68
Lungs	0.00	0.00	0.00
Heart	0.04	0.00	0.00
Intestinal tract with contents	0.05	0.07	0.06
Skin	0.10	0.08	0.00
Remainder	0.13	0.20	0.56
All Tissues	0.32	0.40	1.30

from the blood stream. From other experiments in which only a few tissues were analyzed, it is apparent that from sixteen to seventy-two hours are required to accomplish the result.

Gradual changes in distribution occur between the first and fourteenth days. After that time no obvious evidences of change are found in the analytical results. There is a gradual diminution in the gross amount of lead in the tissues, without any striking shift from one tissue to another. There is a considerable variability among the animals, but the trend is fairly uniform. The bones have absorbed their maximal absolute amounts by the end of two weeks. From this time on, the concentration of lead in the bones does not increase appreciably, either actually or in relation to the other tissues. The spleen apparently loses the greater portion of its lead during the initial two weeks, for large amounts are not found in the spleen of any animal after that time. The liver and the kidneys usually show relatively large amounts of lead. Fatty tissue, striated muscle and intestinal tract (without contents) contain small amounts with considerable regularity. Lead was found in the skin in every analysis, and in amounts which could not be attributed to external contamination. The analysis of the central nervous system did not yield lead regularly. The amounts were small in every instance except one.

Nearly 80 per cent of the animals killed fourteen days or more after the last lead treatment showed measurable amounts of lead in the blood. This fact demonstrates the mobility of lead in the bodies of these animals.

A few findings are not recorded separately. Samples of hair of Rabbits 171, 172 and 206, weighing 11 gm., 11 gm., and 15 gm. respectively, contained 0.00, 0.03 and 0.06 mg. of lead; the testicles, suprarenals, and heart of Rabbit 244, showed respectively 0.06 mg., 0.04 mg., and a trace; the uterus of Rabbit 278 showed a trace, while the suprarenals and heart showed 0.04 and 0.03 mg.; minute amounts of bile from Rabbits 249 and 278 failed to show lead.

The diminution in the actual quantities of lead found in the tissues as a result of excretory processes is best demonstrated by the amounts found in the entire carcasses of Rabbits 242, 244, 250, 255, 206, and 254 (see Table VII, showing the excretory data). It may be noted that after a period of five or six months, there is little evidence of a further drop in the total amount of lead. The explanation of this fact is plain, when reference is made to Tables II and III. An equilibrium had been established with an environment in which lead absorption was unavoidable. We shall return to this point in the data on excretion, but it must be stressed here, that the presence of such amounts of lead, months after its experimental administration, must not be interpreted as evidence of its retention by the tissues. It is the product of the two opposed processes of lead ingestion with food, and lead excretion.

Table IV presents the analytical data on two rabbits which received a suspension of lead orthophosphate intravenously, and on three rabbits similarly injected with secondary lead phosphate. The dosages indicated are approximations. The discrepancies between the recorded dosages and the

TABLE IV

CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAPSE OF TIME FOLLOWING INTRAVENOUS INJECTION OF TERTIARY AND SECONDARY LEAD PHOSPHATES FINELY DIVIDED IN WATER

IDENTIFICATION NUMBER	235	234	239	240	241
Mg. of Pb injected	100	200	100	100	100
Time interval after injection	1 day	3 days	1 day	3 days	37 days
Material injected	Aqueous suspension of $Pb_2(PO_4)_3$		Aqueous suspension of $PbH(PO_4)$		

Lead in Milligrams Found in Tissues

Kidneys	0.03	0.19	0.17	0.31	0.21
Liver	36.00	57.00	33.50	46.50	24.00
Spleen	1.23	7.63	1.70	3.75	5.88
Fat	0.00	0.31	0.05	0.11	0.02
Central nervous system	0.05	0.00	0.00	0.02	0.00
Blood	0.10	0.47	0.30	0.07	0.11
Muscle	0.19	0.11	0.11	0.25	0.14
Bone	1.95	7.00	0.59	3.75	14.50
Washed int. tract	0.03	0.26	0.05	0.14	0.32
All tissues*	39.58	72.97	36.47	54.90	45.18

Lead in Milligrams per 100 Grams of Tissue

Kidneys	0.18	1.36	0.85	1.72	1.75
Liver	46.15	57.00	40.85	44.28	28.57
Spleen	61.50	169.56	42.50	25.00	392.00
Fat		0.37	0.05	0.07	0.04
Central nervous system	0.31			0.12	
Blood	0.08	0.46	0.26	0.05	0.09
Muscle	0.02	0.01	0.01	0.03	0.02
Bone	1.15	4.12	0.33	2.21	10.21
Washed int. tract	0.02	0.10	0.03	0.06	0.14
All tissues*	2.38	4.58	2.42	2.95	3.25

Relative Concentration of Lead in Tissues

Ratio	$\frac{\text{Mg. per 100 Grams Tissue}}{\text{Mg. per 100 Grams All Tissues}}$				
Kidneys	0.07	0.30	0.35	0.58	0.54
Liver	19.36	12.46	16.86	15.02	8.80
Spleen	25.80	37.06	17.54	8.48	120.73
Fat		0.08	0.02	0.02	0.01
Central nervous system	0.13			0.04	
Blood	0.03	0.10	0.11	0.02	0.03
Muscle	0.01	0.01	0.01	0.01	0.01
Bone	0.48	0.90	0.14	0.75	3.14
Washed int. tract	0.01	0.02	0.01	0.02	0.04

*The skin, mixed tissues, and other small tissues not enumerated have been discarded. Therefore the amounts of lead in the entire carcass of the animals are not available.

amounts of lead recovered are explainable on this ground, and for the additional reason that the lungs, skin, feet, ears, and the mixed tissues were not subjected to analysis.

The distribution of the finely divided insoluble lead salts is similar to that of the soluble salts during the first few hours, except that it shows less variation. In every case a large proportion of the lead was recovered from the liver. The amounts in the kidney were small, both in concentration and in the absolute, while the concentration in the spleen was uniformly high. The blood concentration was relatively low and did not obscure the character of

the distribution. (These animals were bled to the point of exsanguination, since none of them died in consequence of the administered lead.)

The time factor in the case of Rabbit 241, gives a special significance to the distribution of lead in its tissues. Thirty-seven days after the injection of secondary lead phosphate, over half of the lead found in the tissues was present in the liver, while less than a third of the recovered lead was found in the skeleton. The total lead recovered is equally remarkable. Little lead had been sufficiently mobile either to redistribute itself into other tissues or to be excreted. The behavior of this lead compound is therefore distinctly different from that of the soluble salts.

The results obtained after the injection of stabilized colloidal lead orthophosphate appear in Table V. Despite the higher dispersion of the particles of the lead compound, no difference in behavior was noted as compared with crystalline suspensions of lead phosphates. A more complete picture of the distribution of lead is presented in that a larger number of tissues appear in the analytical data, but the essential character of the slow migration of lead is unchanged. Again the liver is the chief depository for the injected lead, and again this organ retains a large proportion of its lead for thirty days. The bones slowly gain lead, though it is clear that at some time after the first week they begin to lose it more rapidly than they gain it. It appears that the lungs and the spleen give up all but a small amount of their initial lead, though this apparent trend may be due to the variability in the initial deposition of lead in these tissues. There is no question, however, that the liver and the spleen are more effective filters for the particulate lead than are the lungs and the kidneys.

The study of the activity of colloidal lead brings to light a series of changes in the distribution of lead which resemble those which occur when soluble salts of lead are injected intravenously. Table VI presents the facts. The initial distribution of lead is not remarkable. Rabbits 366 and 365 give a better concept of the early state of affairs than do Rabbits 369 and 368. (Rabbit 369 died from the injection with a high concentration of lead in its blood, while Rabbit 368 was found dead and could not be bled. The blood which was present in the tissues of these animals tends to obscure the picture.) Seven days after the injection, a significant loss of lead from the body had occurred, accompanied by a redistribution of lead in the tissues. Thus the kidneys show little lead, the amounts found in the liver are small in relation to the total lead content of the body, and the spleen shows only traces of lead. The bones, on the contrary, had gained more lead than they had lost. (The amounts of lead recovered from Rabbit 365, as well as the distribution of lead in the tissues, are atypical. This is not chance, but the consequence of lead present in the tissues before the experimental treatment, as shown by the fact that the recovered lead is greater than the amount injected.)

The analytical findings in Rabbit 363 give evidence of further changes in lead distribution. Rabbit 362 undoubtedly had lead in its tissues before treatment, but in addition to this factor its lead excretion was small as compared with Rabbit 363 (Table VII). The facts are evident, nevertheless, when the original amounts in the tissues are obtained by the summation of

TABLE V. CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBIT WITH LAPSE OF TIME FOLLOWING INTRAVENOUS INJECTION OF COLLOIDAL LEAD PHOSPHATE PREPARATIONS IN GELATIN

IDENTIFICATION NUMBER	315	313	318	320	316	356	359	357	360	358
Mg. of Pb injected	±100	±100	±40	±100	±45	7.6	15.2	15.2	15.2	22.8
Time interval after injection	1 hr.	1 day	4 days	1 wk.	1 mo.	3 days	10 days	14 days	30 days	
Material injected	Colloidal $Pb_2(PO_4)_2$ prepared in Lab.					Commercial preparation of colloidal $Pb_2(PO_4)_2$				

Lead in Milligrams Found in Tissues

Kidneys	0.75	0.08	0.09	0.08	0.11	0.00	0.00	trace	0.00	0.00
Liver	23.25	44.25	8.75	50.00	11.50	4.30	4.00	5.20	3.00	3.20
Bile	0.03	0.05	trace	0.00	0.00		0.00		0.00	0.00
Spleen	0.62	2.65	0.21	2.45	0.09	0.20	0.20	0.19	0.68	0.00
Fat		0.03	0.21	0.03	0.00					0.00
Central nervous system	0.19	0.05	0.05	0.03	0.04	0.00	0.00	0.00	0.00	0.00
Blood	33.00	0.07	0.08	0.05	0.04	0.00	0.00	trace	0.00	0.00
Muscle	3.30	0.42	0.24	0.19	0.14	0.05	0.00	0.10	0.00	0.00
Bone	6.20	24.00	16.50	32.00	12.00	0.58	3.80	2.30	1.60	1.90
Lungs	2.22	0.54	0.19	0.40	0.04	0.00	trace	trace	0.00	0.00
Heart	0.44	0.08	0.03	0.04	0.39		0.00	0.00	0.00	0.00
Washed int. tract	0.99	0.34	0.23	0.03	0.12	0.12	0.04	0.16	0.13	0.07
Contents of intestine	0.35	0.90	—*		—*					
Testicles	0.05									
Ovaries		0.07	trace	trace	0.00					
Suprarenals	0.04	0.06	0.04	trace	trace					
Uterus		0.10	0.03	0.00	0.04					
Skin	1.35	—*	0.26		—*	0.12	0.04	0.07	0.00	0.03
Remainder	9.60	6.00	6.00	7.50	1.00	0.28	1.17	1.44	1.28	0.35
All tissues	82.38	79.69	32.95	92.80	25.53	5.65	9.26	9.52	6.69	5.55

Lead in Milligrams per 100 Grams of Tissue

Kidneys	6.25	0.73	0.55	0.62	0.69	0.00	0.00	0.00	0.00	0.00
Liver	27.10	57.50	9.11	43.80	16.20	6.63	4.93	8.14	3.33	3.05
Bile	6.00	3.33			0.00		0.00		0.00	0.00
Spleen	124.00	132.50	7.00	98.00	2.57	10.00	20.00	9.50	68.00	0.00
Fat		0.06	0.15	0.07	0.00					0.00
Central nervous system	1.46	0.44	0.33	0.15	0.21	0.00	0.00	0.00	0.00	0.00
Blood	43.48	0.12	0.07	0.03	0.03	0.00	0.00	0.00	0.00	0.00
Muscle	0.73	0.07	0.04	0.03	0.02	0.01	0.00	0.02	0.00	0.00
Bone	4.37	16.90	11.62	18.83	5.29	0.47	2.31	1.67	0.94	1.19
Lungs	24.67	5.40	1.41	2.86	0.27	0.00		0.00	0.00	0.00
Heart	8.80	1.46	0.43	0.57	4.88		0.00	0.00	0.00	0.00
Washed int. tract	0.58	0.30	0.14	0.02	0.05					
Contents of intestine	0.09	0.53								
Testicles	3.33									
Ovaries		14.00			0.00					
Suprarenals	13.33	12.00	5.00							
Uterus		1.33	0.27	0.00	0.31					
Skin	0.79		0.08			0.07	0.02	0.03	0.00	0.01
Remainder	1.78	0.82	0.52	1.20	0.11	0.04	0.18	0.19	0.13	0.03

Relative Concentration of Lead in Tissues—Ratio

	<i>Mg. per 100 Grams Tissue</i>					<i>Mg. per 100 Grams Total Carcass</i>				
Kidneys	1.17	0.17	0.42	0.14	0.72	0.00	0.00	0.00	0.00	0.00
Liver	5.07	13.50	7.04	10.08	16.91	23.50	11.51	20.42	14.60	17.25
Bile	1.12	0.79			0.00		0.00		0.00	0.00
Spleen	23.20	31.20	5.40	22.50	2.68	35.50	46.95	23.90	298.00	0.00
Fat		0.01	0.11	0.01	0.00					0.00
Central nervous system	0.27	0.10	0.26	0.04	0.22	0.00	0.00	0.00	0.00	0.00
Blood	8.12	0.03	0.05	0.01	0.03	0.00	0.00	0.00	0.00	0.00
Muscle	0.14	0.02	0.03	0.01	0.02	0.04	0.00	0.05	0.00	0.00
Bone	0.82	3.98	8.98	4.33	5.52	1.67	5.52	4.19	4.13	6.73
Lungs	4.60	1.27	1.09	0.66	0.28	0.00	0.00	0.00	0.00	0.00
Heart	1.65	0.34	0.33	0.13	5.09		0.00	0.00	0.00	0.00
Washed int. tract	0.11	0.07	0.10		0.05					
Testicles	0.62									
Ovaries		3.30			0.00					
Suprarenals	2.50	2.82	3.86							
Uterus		0.31	0.21		0.32					
Remainder	0.33	0.19	0.40	0.28	0.11	0.14	0.41	0.47	0.55	0.18

*Discarded. Not included in analytical results.

the lead recovered from the tissues and the excreta. On this basis, Rabbit 362 had 13.7 mg. of lead in its tissues at the outset. This was redistributed in its tissues in substantially the manner shown by other animals of the series, but only 6.45 mg. were excreted. Rabbit 363 had 11.87 mg. originally but in the process of redistribution, it lost 8.88 mg. through excretion.

TABLE VI

CHANGES IN THE AMOUNTS AND CONCENTRATIONS OF LEAD IN THE TISSUES OF RABBITS WITH THE LAPSE OF TIME FOLLOWING INTRAVENOUS INJECTION OF COLLOIDAL LEAD

IDENTIFICATION NUMBER	369	368	366	365	371	370	362	363	372	367
Mg. of Pb injected	12.3	12.3	10.2	12.3	12.3	12.3	12.3	12.3	12.3	12.3
Time interval after injection	25 min.	1 day	3 days		7 days		1 mo.		6 mo.	
<i>Lead in Milligrams Found in Tissues</i>										
Kidneys	0.36	0.39	0.42	0.13	0.07	0.04	0.03	0.00	0.00	0.00
Liver	4.70	2.94	1.50	3.90	1.98	0.90	0.68	0.28	0.00	0.05
Bile			0.00	0.05	trace	0.00	0.00	0.00	0.00	0.00
Spleen	0.24	0.05	0.07	0.05	trace	0.00	0.00	0.00	0.00	0.00
Fat	0.00		0.00	0.00	trace	0.00	trace	0.00	0.00	0.00
Central nervous system	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Blood	0.19	*	0.05	0.04	*	*	0.05	trace	0.00	0.00
Muscle	0.32	0.29	0.00	0.07	trace	trace	0.09	0.09	0.04	0.00
Bone	2.16	1.35	1.62	5.60	2.60	4.20	5.20	1.38	1.50	1.74
Lungs			0.09	0.15	0.06	trace	0.00	0.00	trace	0.00
Heart			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Int. tract and contents	0.81	1.92	1.11	2.16	0.90	1.29	0.10	0.08	0.13	0.32
Skin	†	†	†	†	†	†	0.09	0.15	0.00	0.09
Remainder	0.96	0.96	0.54	1.21	0.76	0.96	2.00	0.99	0.78	0.03
Entire carcass	9.74	7.90	5.40	13.36	6.37	7.42	8.25	2.99	2.46	2.25

Lead in Milligrams per 100 Grams of Tissue

Kidneys	2.25	2.29	2.21	0.72	0.31	0.27	0.16	0.00	0.00	0.00
Liver	5.87	2.69	2.73	4.70	1.86	1.33	0.78	0.44	0.00	0.05
Bile			0.00	10.00		0.00	0.00	0.00	0.00	0.00
Spleen	8.00	2.50	3.50	5.00		0.00	0.00	0.00	0.00	0.00
Fat	0.00		0.00	0.00			0.00		0.00	0.00
Central nervous system	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Blood	0.54		0.08	0.05			0.05		0.00	0.00
Muscle	0.08	0.07	0.00	0.02	0.00		0.01	0.02	0.01	0.00
Bone	1.22	0.58	1.00	4.79	1.54	2.33	2.48	0.75	0.65	1.05
Lungs			0.10	1.36	0.35		0.00		0.00	0.00
Heart			0.00	0.00		0.00	0.00	0.00	0.00	0.00
Int. tract and contents	0.21	0.33	0.38	0.67	0.29	0.46	0.02	0.02	0.02	0.08
Remainder	0.11	0.12	0.07	0.22	0.10	0.13	0.21	0.10	0.05	0.01-

Relative Concentration of Lead in Tissues—Ratio

	<i>Mg. per 100 Grams Tissue</i>									
	<i>Mg. per 100 Grams Total Carcass</i>									
Kidneys	4.63	6.42	8.53	0.91	0.94	0.62	0.58			
Liver	12.07	7.52	10.50	5.92	5.53	3.11	2.87	4.16		0.79
Bile				12.64						
Spleen	16.45	6.97	13.52	6.32						
Fat										
Central nervous system										
Blood	1.12		0.31	0.06			0.17			
Muscle	0.16	0.19		0.02			0.05	0.14	0.09	
Bone	2.51	1.62	0.40	6.02	4.60	5.48	9.12	7.03	11.21	15.64
Lungs			0.40	1.72	1.06					
Heart										
Int. tract and contents	0.44	0.93	1.45	0.85	0.88	1.07	0.08	0.18	0.40	1.16
Remainder	0.23	0.33	0.25	0.27	0.30	0.31	0.76	0.92	0.78	0.03

*No blood sample available by reason of death of animal.

†Skin and ears discarded.

TABLE

LEAD EXCRETION OF RABBITS FOLLOWING INTRAVENOUS

AQUEOUS SOLUTION OF $PbCl_2$										COMMERCIAL
SUCCESSIVE DAYS EXCRETION	242	244	255	250	254	249		278		SUCCESSIVE DAYS EXCRETION
	12 MG. Pb IN 2 DOSES 1 WK. APART AS INDICATED BY*. KILLED AT END	4 MG. AND 6 MG. Pb 1 WK. APART AS INDICATED BY*. KILLED AT END	12 MG. Pb IN 2 DOSES 1 WK. APART AS INDICATED BY*. KILLED AT END	12 MG. Pb IN 2 DOSES 1 WK. APART LAST DOSE 1 MONTH BEFORE EXCRE- TORY PE- RIOD, KILLED AT END	12 MG. Pb IN 2 DOSES 1 WK. APART 6 MO. BEFORE EXCRE- TORY PE- RIOD, KILLED AT END	12 MG. AND 6 MG. Pb 3 MO. APART. LAST INJEC- TION 11 MO BEFORE EX- CRETORY PE- RIOD, KILLED AT END		6 MG. Pb IN 1 DOSE 11 MO. BEFORE EXCRETORY PERIOD, KILLED AT END		
	LEAD IN MG.	LEAD IN MG.	LEAD IN MG.	LEAD IN MG.	LEAD IN MG.	LEAD IN MG.		LEAD IN MG.		
	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	COMBINED FECES URINE	FECES	URINE	FECES	
2	Lost	(0.61)†								4
1	0.67*	0.44*	0.41*							4
1	0.48	0.39	0.24							4
1	0.72	0.73	0.19							4
1	0.41	0.67	0.15							4
3	0.58	0.91	0.35							4
1	0.60*	0.75*	0.32*							4
1	0.62	0.95	0.67							4
1	0.27	0.99	0.28							
1	0.25	0.99	0.25							
4	0.17	0.46	0.38							
4	0.12	0.71	0.09							
3 (2 No. 242)	0.27	0.11	0.31							
4		0.27	0.04							
4		0.24	0.09							
4		0.28	0.00							
1 (1 No. 244)		0.47	0.00							
4			0.07	0.32	0.31	0.21	0.03	0.21	0.03	
4 (5 No. 254)			0.31	0.50	0.39	0.11	0.05	0.20	0.02	
4 { 3 No. 250 }										
4 { 3 No. 255 }			0.27	0.46	0.17	0.24	0.05	0.23	0.00	
Total excretion	5.16	9.36	4.42	1.28	0.87	0.56	0.13	0.64	0.05	
Lead in carcass in mg.	5.91‡	5.10	3.77‡	3.82	1.72	2.33		3.33		
Average First 4 days	0.57	0.56	0.25							
Entire excretion period	0.25	0.27	0.09	0.12	0.07	0.06		0.06		

†Not included in total excretion.

‡Including contents of alimentary tract not shown in table.

The analytical results on the animals which were sacrificed at the end of six months are like those shown after a corresponding interval in Table I.

The analytical data obtained from the study of lead excretion following intravenous administration of lead compounds, are shown in Table VII. The excretory response of the animals treated either with lead chloride or colloidal lead was prompt and definite. The two series of results are not directly comparable, because the urine and feces were not collected separately in the case of the animals treated with lead chloride, but they are of the same general order of magnitude. (For reasons which will appear later, the results on the lead chloride animals must be regarded as merely indicative of a trend which is not necessarily accurate in its details.) In sharp contrast, the lead excretion of the animals treated with colloidal lead orthophosphate showed little or no increase. A slight elevation of the excretory rate in the feces appears to have resulted, if the findings are compared with those obtained on normal rabbits, while the urinary lead, though slightly higher than

VII

ADMINISTRATION OF VARIOUS LEAD COMPOUNDS

PREPARATION OF COLLOIDAL $Pb_3(PO_4)_2$						COMMERCIAL PREPARATION OF COLLOIDAL Pb											
361		359		360		364		362		363		373		372		367	
CONTROL. NO TREATMENT. KILLED AT END OF EXCRETORY PERIOD		15.2 MG. Pb AT START OF EXCRETORY PERIOD. DIED 15TH DAY		15.2 MG. Pb AT START OF EXCRETORY PERIOD. KILLED 32ND DAY		CONTROL. NO TREATMENT		12.5 MG. Pb AT START OF EXCRETORY PERIOD. KILLED 28TH DAY		CONTROL. NO TREATMENT. KILLED AT END OF EXCRETORY PERIOD		12.5 MG. Pb 6 MO. BEFORE EXCRETORY PERIOD. KILLED AT END OF EXCRETORY PERIOD					
LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.		LEAD IN MG.	
FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE	FECES	URINE
0.20	0.01	0.48	0.07	0.53	0.00	0.21	0.05	1.66	0.32	3.50	0.60						
0.18	0.00	0.38	0.09	0.39	0.07	0.19	0.04	0.91	0.22	1.50	0.17						
0.16	0.00	0.14	0.00	0.36	0.02	0.26	0.03	0.42	0.21	0.84	0.15						
0.20	0.01			0.36	0.05	0.05	0.03	0.57	0.18	0.47	0.28						
0.20	0.00			0.30	0.02	0.19	0.03	0.34	0.13	0.42	0.12						
0.26	0.01			0.30	0.03	0.16	0.06	0.17	0.00	0.25	0.17	0.19	0.10	0.51	0.05	0.43	0.02
0.19	0.01			0.20	0.04	0.12	0.04	0.18	0.14	0.28	0.13	0.23	0.00	0.30	0.05	0.31	0.03
0.19	0.03			0.50	0.00												
1.58	0.07	1.00	0.16	2.54	0.23	1.18	0.29	4.25	1.20	7.26	1.62	0.42	0.10	0.81	0.12	0.74	0.05
1.30		9.26		6.69		0.32		8.25		2.99		0.40		2.46		2.25	
0.05		0.14		0.08		0.07		0.49		1.02							
0.05		0.09		0.09		0.05		0.19		0.32		0.07		0.12		0.08	

that of the control animal studied at the same time (Rabbit 361), is little above that of normal animals observed on other occasions (e.g., Rabbits 364 and 373).*

These observed facts are in harmony with expectations based on the chemical properties of the several lead compounds, and they are in accord with the result obtained by Bischoff⁴ and his associates in their determination of the toxicity of lead orthophosphate, colloidal lead and certain soluble lead salts.

*Fecal lead output cannot be regarded as an expression of the excretory activity of the tissues because of the variability in the lead content of ingested food. Practically all food substances contain small amounts of lead, most of which is unabsorbed. Contrary to general opinion as expressed in experimental procedures, such lead occurs not in traces, but in readily measurable amounts of high variability. For this reason, we must rely mainly upon the urinary lead excretion for the interpretation of these experimental results, although we may use the alimentary lead excretion of control animals as a means of estimating the approximate magnitude of the ingested lead. The recognition of these facts has led us to separate the urinary and feces in all our recent animal experiments and to base our conclusions as to excretory processes chiefly on the urinary findings. The work on lead chloride was nearly completed before we became aware of the importance of the lead in food materials.

The findings in the tissues and in the excreta of human subjects who were treated with colloidal lead compounds are essentially like those obtained in the animal experiments. Table VIII gives the distribution of lead in the tissues of a patient treated for carcinoma of the cervix uteri by the intravenous administration of colloidal tri-lead phosphate. The last dose was administered six days before death. A comparison of the distribution of lead in the tissues of this patient with that found in a rabbit sacrificed one week after similar treatment, shows a high degree of correspondence. A large proportion of the lead was found in the liver, and relatively little in the skeleton, despite the time elapsing between the administration of the first two doses of lead and the death of the patient. Obviously the transfer of lead from

TABLE VIII

DISTRIBUTION OF LEAD IN HUMAN TISSUES AFTER INTRAVENOUS ADMINISTRATION OF COMMERCIAL PREPARATION OF COLLOIDAL LEAD PHOSPHATE

PATIENT A. A. WITH CARCINOMA OF CERVIX UTERI, TREATED WITH 68.4 MG. PB AS COLLOIDAL LEAD PHOSPHATE, 7.6 MG. 3/10/31, 15.2 MG. 3/14/31, AND 45.6 MG. 3/31/31. DIED 4/6/31

TISSUE	WEIGHT IN GM.	LEAD FOUND IN MG.	LEAD IN MG. PER 100 GM.	RATIO: $\frac{\text{MG. PER 100 GM. OF TISSUE}}{\text{MG. PER 100 GM. OF ENTIRE BODY}}$
Kidneys	250.0	0.08	0.03	0.21
Liver	1220.0	17.00	1.39	9.93
Spleen	88.0	3.28	3.73	26.65
Fat	185.0	0.00	0.00	
Muscle	25.5	0.01	0.04	0.28
Bone (flat)	29.2	0.15	0.51	3.65
Lungs	1100.0	0.41	0.04	0.28
Heart	290.0	0.00	0.00	
Washed int. tract	1292.0	0.08	0.01-	0.04
Pancreas	160.0	0.12	0.08	0.57
Cancer tissue	235.0	0.04	0.02-	0.12
All tissues	4904.7	21.17	0.43*	

*This figure is faulty because of the small amounts of bone and muscle subjected to analysis. Calculating the total lead in the body on the basis of estimates of the total weights of bone and muscle (approximately 8 and 20 kilograms respectively) the total lead approximates 70 mg. Pb. in a body of 50 kilograms of estimated weight. The gross concentration thus becomes approximately 0.14 mg. Pb per 100 grams. The ratio is calculated on this figure.

the liver (and the spleen) into the bones had been taking place slowly. Furthermore, little had been excreted. The calculation of the total lead in the body is somewhat inaccurate because the actual weights of bone and muscle were not available, and the bones which were analyzed (ribs and sternum) may not have been representative of the lead content of the skeleton in general. Despite these considerations there is no doubt of the essential correctness of the picture when the findings and calculations are compared with those which appear in Table IX. The latter table (IX) shows the results obtained in the analysis of the tissues of a patient whose death from carcinoma of the breast occurred seventeen days after a single intravenous injection of colloidal lead. There are certain differences in the distribution of lead in the tissues in the two cases, viz.; the patient treated with colloidal lead shows a higher concentration of lead in the kidneys, muscle, and heart muscle, a lower concentration of lead in the liver, spleen and bones, and a smaller total amount of lead in the combined tissues. Evidently lead has migrated more rapidly and more generally in the body of the patient treated with col-

TABLE IX

DISTRIBUTION OF LEAD IN HUMAN TISSUES AFTER INTRAVENOUS ADMINISTRATION OF COMMERCIAL PREPARATION OF COLLOIDAL LEAD

PATIENT E. J. WITH CARCINOMA OF BREAST, TREATED WITH 82 MG. PB AS COLLOIDAL LEAD IN ONE DOSE. DIED SEVENTEEN DAYS LATER

TISSUE	WEIGHT IN GM.	LEAD FOUND IN MG.	LEAD IN MG. PER 100 GM.	RATIO: $\frac{\text{MG. PER 100 GM. OF TISSUE}}{\text{MG. PER 100 GM. OF ENTIRE BODY}}$
Kidneys	330.0	0.96	0.29	3.22
Liver	3820.0	26.08	0.69	7.67
Spleen	233.0	1.35	0.58	6.44
Fat	365.0	0.16	0.04	0.44
Central nervous system	1312.0	2.03	0.15	1.67
Muscle	172.5	0.08	0.05	0.55
Bone	442.0	0.74	0.17	1.89
Lungs	560.0	0.07	0.01	0.11
Heart	302.0	0.30	0.10	1.11
Thyroid	18.5	0.03	0.16	1.78
Suprarenals	19.0	0.02	0.11	1.22
Uterus	70.0	0.02	0.03	0.33
Hypophysis	1.0	0.00	0.00	
Pancreas	58.5	0.16	0.28	3.11
Cancer tissue	445.0	0.35	0.08	0.89
All tissues	8148.5	32.35	0.40*	

*Correction of this figure must be made as in Table VIII. On the basis of total weights of fat, bone and muscle (approximately 11, 9 and 24 kilograms respectively) the total lead in the body was approximately 64 mg. In the body (approximately 70 kilograms) the calculated concentration would be 0.09 mg. Pb per 100 grams. The figures in the last column are based on this calculated concentration.

TABLE X

DISTRIBUTION OF LEAD IN TISSUES OF NORMAL HUMAN ADULT

YOUNG HEALTHY MALE NEGRO, WITH NO HISTORY OF LEAD EXPOSURE, KILLED BY STAB WOUND OF HEART

TISSUE	WEIGHT IN GM.	LEAD FOUND IN MG.	LEAD IN MG. PER 100 GM.	RATIO: $\frac{\text{MG. PER 100 GM. OF TISSUE}}{\text{MG. PER 100 GM. OF ENTIRE BODY}}$
Kidneys	300.0	0.21	0.07	0.41
Liver	2073.0	1.75	0.08	0.46
Spleen	173.5	0.00	0.00	
Fat	109.0	0.00	0.00	
Muscle	388.0	0.00	0.00	
Bone (flat)	92.8	1.03	1.11	6.53
Cartilage	10.6	0.00	0.00	
Lungs	786.0	0.00	0.00	
Heart	276.5	0.00	0.00	
Thyroid	41.5	0.00	0.00	
Suprarenals	10.3	0.00	0.00	
Pancreas	106.7	0.04	0.04-	0.23
Prostate	5.8	0.00	0.00	
Bladder	79.3	0.00	0.00	
Portion spinal cord and femoral nerve	17.8	0.00	0.00	
All tissues	4470.2	3.03	0.07*	

*This figure is corrected as in Tables VIII and IX in terms of total weight of bone (12 kilo.) and total weight of the body (80 kilo.). The total lead comes to approximately 135 mg., giving a concentration of 0.17 mg. per 100 gm. of the entire body. The ratios recorded in the last column are calculated on this basis.

loidal lead than in that of the patient treated with colloidal lead phosphate. It should be noted that there was no selective absorption of lead in the tissue of the tumor in either instance.

The types of distribution of lead shown in Tables VIII and IX are different from each other. Yet both are distinctly unlike that which is found when the usual type of lead absorption, over a period of time, has taken place. Undoubtedly, the character of the distribution of lead in human tissues may be found to vary greatly with a number of factors. Nevertheless when a condition of approximate equilibrium with respect to a prolonged and regular lead absorption has been brought about, the end-result is similar to that which is illustrated in Table X. The data presented in the table were obtained from the analysis of the tissues of a healthy young man who died immediately after a stab wound of the heart. His occupational history gave no evidence of recent industrial exposure to lead.

In Table XI the urinary excretion of lead following the injection of colloidal lead phosphate and colloidal lead are compared. Case A. A. showed no appreciable increase in urinary lead output following the administration of 3 doses totaling 68.4 mg. of lead as colloidal lead phosphate. This fact coincides with the data of Table VIII which show that most of the adminis-

TABLE XI

URINARY LEAD EXCRETION OF HUMAN SUBJECTS FOLLOWING INTRAVENOUS ADMINISTRATION OF COLLOIDAL LEAD COMPOUNDS

COLLOIDAL LEAD PHOSPHATE					COLLOIDAL LEAD				
SUCCESSIVE DAYS' EXCRETION	PATIENT A. A.		PATIENT B. L.		SUCCESSIVE DAYS' EXCRETION	PATIENT P. B.		PATIENT H.	
	LEAD IN MG.		LEAD IN MG.			LEAD IN MG.		LEAD IN MG.	
	FOUND	PER LITER	FOUND	PER LITER		FOUND	PER LITER	FOUND	PER LITER
Control					Control				
2 days	0.02	0.02	0.06	0.05	2 days			0.17	0.11
	7.6 mg. Pb as $Pb_3(PO_4)_2$					49.2 mg. Pb		45.1 mg. Pb	
1 day	0.00	0.00	0.00	0.00	3 days	0.70	0.65	0.02	0.01
1 day	0.03	0.02	0.00	0.00	3 days	0.01	0.01	0.43	0.19
1 day	0.02	0.02	0.00	0.00		82 mg. Pb		71.75 mg. Pb	
1 day	0.00	0.00	0.03	0.02	3 days	0.55	0.42	1.44	0.38
	15.2 mg. Pb as $Pb_3(PO_4)_2$				3 days	0.49	0.28	0.74	0.19
3 days	0.07	0.02	0.07	0.07		41 mg. Pb		57.4 mg. Pb	
3 days	0.04	0.01	0.17	0.14	3 days	0.31	0.17	0.93	0.31
3 days	0.00	0.00	0.21	0.16	3 days	0.35	0.26	0.92	0.34
3 days	0.02	0.01-	0.12	0.11	3 days	0.36	0.19	0.64	0.22
3 days	0.01	0.01-	0.08	0.06	3 days	61.5 mg. Pb		0.30	0.07
	45.7 mg. Pb as $Pb_3(PO_4)_2$				3 days	0.50	0.20	0.27	0.22
3 days	0.05	0.02	0.10	0.09	3 days	0.40	0.15		
3 days			0.28	0.22		45.1 mg. Pb			
2 days			0.28	0.23	3 days	0.44	0.25		
3 days			0.31	0.28	3 days	0.40	0.17		
	95 mg. Pb as $Pb_3(PO_4)_2$				3 days	0.38	0.25		
3 days			0.24	0.19	3 days	0.00	0.00		
4 days			0.38	0.28	3 days	0.29	0.10		
2 days			0.41	0.29	3 days	0.42	0.18		
3 days			0.48	0.32	3 days	0.19	0.19		
2 days			0.18	0.24					
3 days			0.34	0.23	3 days	0.27	0.15		
2 days			0.12	0.17	3 days	0.20	0.12		
3 days			0.31	0.24	2 days	0.10	0.05		
4 days			0.57	0.34	3 days	0.17	0.04		
4 days			0.11	0.07	2 days	0.22	0.06		

tered lead was retained in the tissues. Case B. L. on the other hand, showed a doubtful increase in urinary lead excretion after 22.8 mg. of colloidal lead phosphate had been injected, a definite response after a total dosage of 68.4 mg. had been given, and a still further increase after the gross dosage of 163.4 mg. had been reached. Both patients who were treated with colloidal lead showed a quicker and greater elevation of urinary lead excretion than that seen in the patients who received colloidal lead phosphate. The rate of urinary excretion after the administration of colloidal lead phosphate rises slowly but steadily with each additional increment of injected lead, showing little tendency to diminish between doses. The opposite result occurs after the administration of colloidal lead. A rapid rise in the excretory rate is followed by a rapid fall in most instances. However, the rate after the initial drop is usually well above the level shown by untreated or unexposed persons. These facts are in accord with the other evidences that colloidal lead phosphate is relatively inert in the body while colloidal lead is a comparatively active compound.

DISCUSSION

The foregoing experimental observations make it apparent that the relatively insoluble tertiary and secondary phosphates of lead are chemically and physiologically inert as compared to the compounds formed in the blood when soluble salts of lead, and colloidal lead are injected intravenously. This proves that soluble lead salts are not converted quantitatively into inorganic lead phosphates when introduced into the blood stream. The conclusion that lead is carried in the blood in the form of inorganic phosphates, which was arrived at by Aub²⁰ and his associates, and by Brooks,²¹ after experimentation *in vitro*, is therefore untenable.

The results obtained on animals treated with colloidal lead show that this compound of lead is almost as reactive in the body as the water soluble salts of lead. Thus it seems probable that colloidal lead is rapidly converted into the same compounds which are formed when lead salts are injected intravenously. This conclusion is in agreement with the experimental observations of Bischoff and Blatherwick,²¹ on the toxicity of colloidal lead, and on its chemical instability under conditions similar to those which exist in the blood stream.

The usefulness of the foregoing studies to those who are employing lead compounds in the treatment of malignant tumors, will depend chiefly upon a point of view as to the mode of action of lead therapeutically. If a maintained lead stream of low concentration will combat malignant proliferation, then the use of large doses of lead orthophosphate is justified. On the other hand, if the effect of lead upon tumor tissue depends upon the maintenance of a high concentration of chemically reactive lead in the blood or in the tumor, compounds of lead which are less inert than lead phosphate should be employed. The use of such compounds will certainly give rise to danger in that general lead intoxication in the patient may occur simultaneously with the toxic effect upon the tumor cells, despite the fact that the latter may be more susceptible than the normal tissues.

The hope of obtaining selective localization of lead in tumor tissues, through use of the preparations here studied, is small. The spleen and liver, as well as the lungs, may be expected to remove the greater proportion of lead from the circulation, and the originally high concentrations of lead in these tissues will diminish rapidly (except after the use of the relatively inert lead phosphates) through the migration of the lead into the skeleton, and through excretion from the body.

Finally, it needs to be reemphasized that lead compounds are not stored in living tissues in inert form. Lead continues to be excreted from the tissues until there is a balance between that which is being excreted and that which is being absorbed as the result of unavoidable contacts with lead in food materials and elsewhere. Thus it appears that the mechanisms of lead deposition and metabolism in the tissues are not different in principle from those of the common mineral elements of the body.

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LABORATORY METHODS

AN ADAPTATION OF SAND FILTRATION TO THE RAPID CLEARING OF HEAVY BACTERIAL CULTURE MEDIA

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THE appliance pictured and described below was perfected by the author, with the assistance of his laboratory technicians, and has been used over a period of fifteen years with considerable satisfaction.

The appliance consists of (1) a filtering unit, (2) a collecting bottle, and (3) a vacuum (pressure) pump of sufficient power to produce a negative pressure of at least twenty pounds in a collecting jar of 4,000 c.c. capacity.

The filter consists of a one-gallon glass percolator, the lower end of which is calibrated to accommodate a one-fourth inch heavy walled rubber tube, of sufficient length to reach to the collecting bottle. In the bottom of this percolator is placed a perforated porcelain disc, such as is ordinarily used in a chemical laboratory and of a size that fits snugly. Immediately over this is placed a layer of medium coarse glass wool, two inches thick, and upon this five inches of hot, wet, cleaned sand of a size which will pass a No. 60 mesh screen but be retained by a No. 80 mesh screen.

The culture medium to be cleared, such as nutrient agar-agar or gelatin, is poured, while quite hot, directly upon this layer of sand.

The collecting bottle is equipped with a tightly fitting rubber stopper, with two perforations, through each of which is placed a tightly fitting glass tube about three inches long. To one of these two tubes is connected the rubber tube attached to the percolator; to the other is attached a similar heavy walled rubber tube, the free end of which is attached to the negative pressure pump.

After the pump (which may be electric, or water or hand powered) is started and a negative pressure is produced in the collecting bottle, the culture medium is drawn through the filter and to the collecting bottle, at a rather rapid rate and sufficiently clear for all ordinary bacteriologic work. If egg white is added to the medium to be cleared, the fluid delivered is clearer; the surface of the sand filter may become clogged, however, requiring that it be broken by scraping the surface of the sand with some instrument, such as a heavy stirring rod, introduced through the layer of culture medium over the sand; with which the surface is forcibly broken. This may be repeated several times, if necessary, without interfering with the efficiency of the filter.

The filter has the advantage of filtering sufficiently clear for all ordinary bacteriologic work any of the heavy culture media, including those impaired by contact with organic filter substances, such as Huntoon's Hormone Medium. The media are cleared rapidly, with practically no loss, as the filter may be run until all of the medium is drawn through the filter layers.

The filtered culture medium is unchanged chemically, as it comes in contact with no organic substance with the exception of the rubber tube connecting the

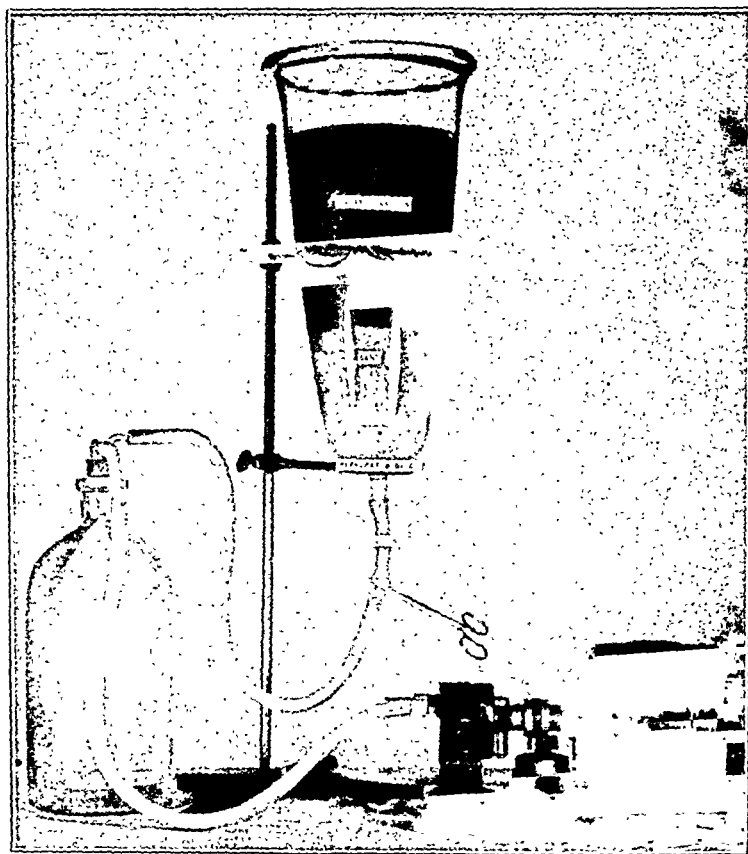


Fig. 1

percolator with the collecting bottle. In our experience this tube has caused no change. If desired, however, this connection may be made with glass tubing.

The filter may be taken down immediately after using, while still hot. The glass wool and porcelain disc may be washed in several changes of boiling water. The sand may likewise be cleaned and used again if desired.

This is the apparatus as used by us in the Bacteriological Laboratory at the Medical College of the State of South Carolina. Its size, and the amounts and sizes of the filtering materials may be changed to suit unusual conditions.

A SIMPLE AND EFFICIENT ELECTRICAL TOOL FOR SEALING MUSEUM JARS*

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THE use of an asphalt cement for sealing museum jars and cells in which pathologic specimens are mounted, while comparatively recent, has proved to be so satisfactory^{1, 2} that such a cement is rapidly supplanting those composed of other constituents, especially resins, waxes, and oxides.

Experience with several types of cement, including some of those which are applied cold, indicates that cements containing asphaltum as the principal constituent give the most favorable results and meet nearly all conditions very satisfactorily. When, however, Judah's modification of Kaiserling's solution³ is used for the final preserving medium there is a tendency for the cement to become liquefied, possibly owing to the liberation of free chloroform from the chloral hydrate which is one of the constituents of the solution. Specimens preserved in solutions without chloral hydrate and sealed with the same cement have not shown a tendency toward liquefaction.

The usefulness of a cement for specimen jars depends on its insolubility, adhesiveness, tenacity, and, in its final physical state, on its plasticity. Cements reach their permanent state by evaporation of the solvent, if used, by oxidation, by a physical change upon cooling, or by a combination of two, or of all three of these processes, depending on the constituents of the original cement. It is obvious that the more quickly the final set is reached the more adapted is the cement for wet tissue mounts.

As asphaltum meets the requirements of a suitable sealing substance, the electrical tool here illustrated and described has been designed for the rapid and efficient application of cements containing it.

The applicator is suitable for sealing practically any type of museum jar and is especially useful for applying asphalt around the cell for thin pathologic mounts as prepared by Davis.⁴

Its advantages are: that it permits quicker and neater work than the usual method, does away with the use of a sand bath for heating, and, to some extent, with disagreeable odors; it is always ready for use, and can be kept in any drawer or receptacle with other tools. Further, one or many containers can be sealed at any time without the usual preliminary preparation or manipulation of the cement. The rectangular jar is prepared in the usual manner; the surface being cleaned first with soap and water, then with absolute alcohol and benzol. It is heated with a flame until hot to the touch, the cement is applied, and the previously heated lid put on and weighted, to insure a thin joint, thus minimizing the amount of surface which can be acted upon by the preserving solution.

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Manipulation of the applicator is as follows: The reservoir is filled from the stock container with liquefied cement to a level just above the sluice leading to the point; the apparatus is then tilted back far enough to prevent the cement from flowing out. The current is turned on and the cement allowed to come nearly to the boiling point. The tip of the applicator is then drawn along the top of the jar near the outer edge and the required amount of cement, which can be regulated by the speed at which the applicator is moved, is thus deposited on the surface to be sealed. An excess of cement, or applica-

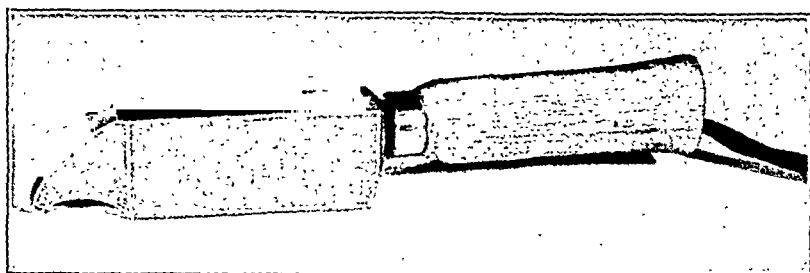


FIG. 1

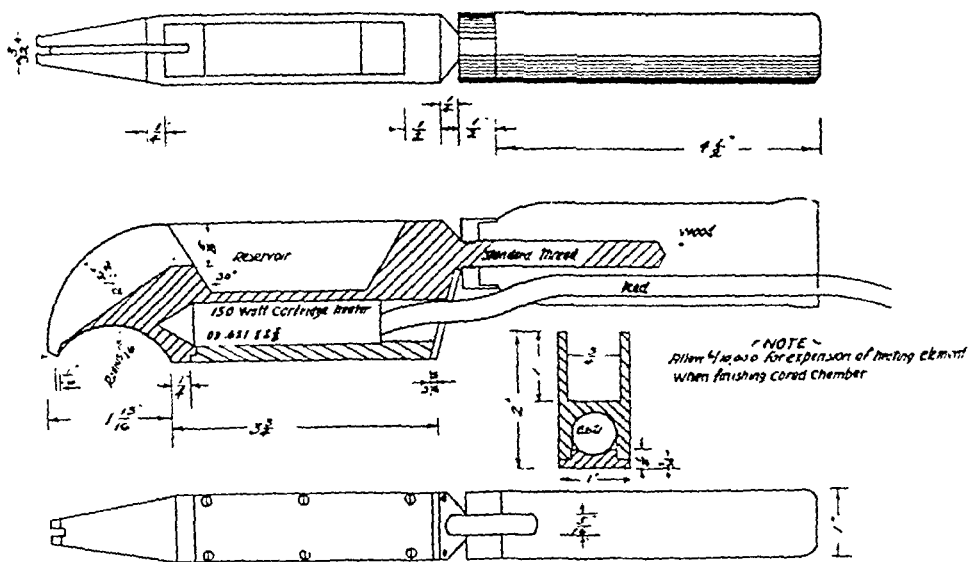


FIG. 2

tion too near the inner edge, may cause superfluous material to be squeezed into the jar when the lid is pressed down. A little practice is needed to gauge the speed and angle at which to operate most satisfactorily.

DESCRIPTION OF THE APPARATUS

The apparatus is 1 by 2 by 11 inches over all and weighs one pound. It is composed of a body, which contains the heating element, a tip or applying point with a sluice connecting with the reservoir for the cement, and an operating handle of wood (Fig. 1). The applicator is milled from a cored alumi-

mium casting, the dimensions of which are shown on the plan (Fig. 2). The casting was made in a commercial foundry, the shaping, finishing, and electrical work being done in the laboratory machine shop. The heat is supplied by a 150 watt cartridge heating unit placed well forward under the reservoir to insure adequate heating of the point. Such heating units operate on 110 volts, 60 cycle alternating current. As may be noted on the drawing, the applicator base, which is of brass, is removable, in order to facilitate the machine work in the heating chamber. The greatest efficiency in heating is obtained when the unit is in contact with the surrounding metal, but, owing to the difference in the expansion of the metals, a clearance of $\frac{1}{10,000}$ of an inch must be allowed when the chamber is milled. The lead should be asbestos wound and not rubber coated, as there is more heat in the chamber and handle than rubber will withstand.

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A SIMPLE AUTOMATIC SHARPENER FOR MICROTOME KNIVES*

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THERE can be few histologists who have not hoped to find a more efficient method of sharpening microtome knives than the usual hand honing and stropping. The need has been partially supplied by the automatic machines of Fanz,¹ but they are open to two objections. The cost of the apparatus, especially of the machine for larger knives, is prohibitive for many laboratories, and the knife blade is subjected to more wear at the edge of the disc than near the center. The device to be described can be assembled for less than thirty dollars from materials available at any large hardware store, and can easily be adapted for knives of any size.

The principle employed was suggested to me by Dr. Samuel Orton, who built a somewhat similar apparatus, and I most gratefully acknowledge my indebtedness to him. My own additions have been purely mechanical ones.

Sharpening is carried out by resting the edge of the knife against a revolving fiber cylinder (Fig. 1, A). The cylinder consists of a "Rockwood" fiber pulley, 9 inches in width and 8 inches in diameter, which has been turned down in a lathe to an absolutely true surface. These pulleys are made for 2 inch shafting, which is too large for convenient use. A $\frac{1}{2}$ inch shaft,

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bearing two 2 inch pulleys as bushings, is inserted in the bore of the large pulley, and is quite strong enough to carry the weight involved. The shaft in turn revolves upon two bronze pillow blocks.

The cylinder is turned by a $\frac{1}{4}$ h. p. motor by means of a V belt. A motor pulley of 2 inches and a shaft pulley of 8 inches provide a suitable reduction.

If the knife is merely laid on the cylinder, it inevitably becomes grooved by irregularities of the surface. It must be given a slight lateral motion, of about an inch in each direction. This is accomplished by attaching the knife holder to a $\frac{1}{2}$ inch shaft which slides back and forth in pillar bearings, parallel to the cylinder (Fig. 1, *B*). The reciprocating motion is obtained from a crank actuated by a bevel gear lying in the plane of the base of the machine,

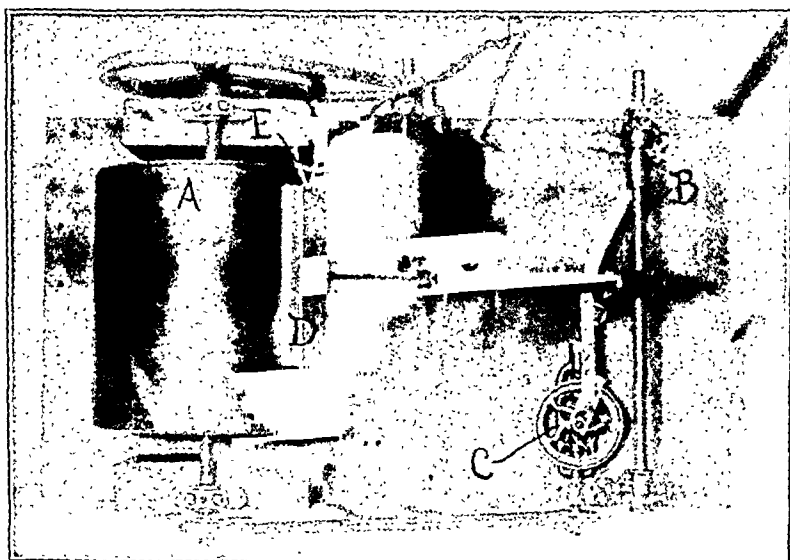


Fig. 1.—Knife sharpener seen from above. *A*, Polished fiber cylinder; *B*, reciprocating shaft; *C*, reciprocating mechanism made from a hand drill; *D*, knife holder; *E*, "filler" fitting inside knife holder to adapt it to knives of different sizes.

and actuated by a small belt and pulley from the cylinder shaft. A cheap hand drill furnishes bevel gears of a convenient size (Fig. 1, *C*).

The knife holder itself is the only part of the apparatus which cannot be purchased ready for use. It consists of a square rod of metal the length and thickness of the back of the largest knife to be sharpened, to which are riveted two flat pieces of spring bronze of equal length and wide enough to hold the knife firmly without striking the cylinder; that is, the edge of the holder comes within about half an inch of the edge of the blade. The bronze strips are bent toward each other so as to hold the knife firmly (Fig. 1, *D*). If smaller knives are to be sharpened also, a small metal "filler" is used, which fits inside the holder and holds the edge of the small knife at a suitable angle to the surface of the cylinder (Fig. 1, *E*). The knife holder swivels on a rod rigidly attached at right angles to the reciprocating shaft. The distance from the reciprocating shaft to the holder is adjustable so that the optimum angle

for the edge of the knife may be found by trial. A weight of about a kilogram should be placed on the rod close to the knife holder to increase the pressure of the blade against the strap (not shown in the illustration).

The only abrasive which is safe to use with this apparatus is jewelers' rouge, made into a thin suspension with castor oil. Nicks no deeper than the honed edge may be removed in ten hours' grinding or less, and no deep grooves are cut. The rouge should be employed as little as possible, however, and carefully washed off with soap and water after use, as a better polish may be obtained with no abrasive at all. A mixture of equal parts of linseed oil and turpentine with a little Japan dryer makes a good dressing and the cylinder gradually acquires an "oil polish." Great care should be taken to keep it clean. It would doubtless be easy to prepare a second cylinder to be used with emery, but this has not proved necessary so far.

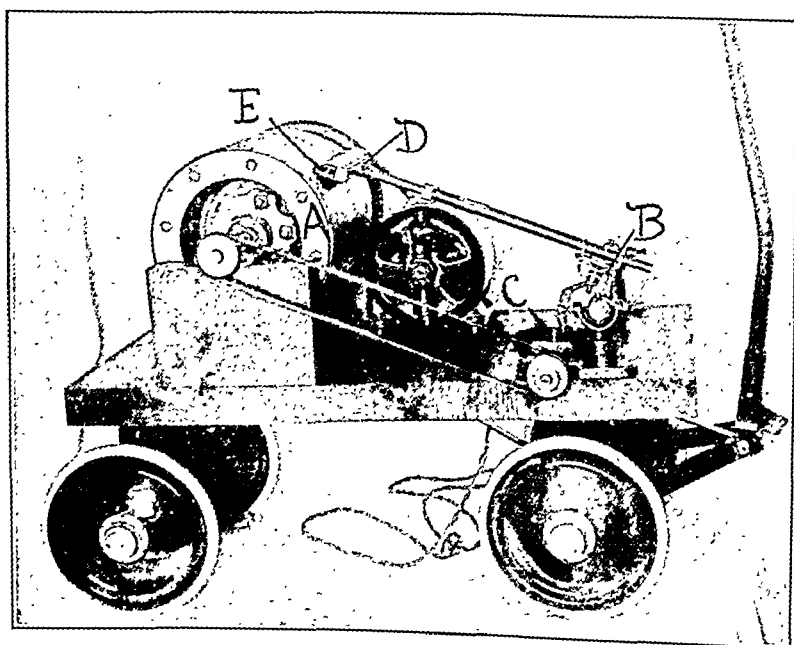


Fig. 2.—Side view of machine. Parts lettered as in Fig. 1.

The angle of the knife should be so adjusted that the polished edge is about 0.5 mm. wide. If it is much wider, a trying "false edge" will form. As the edge is slightly concave, it is sharper than one of similar width produced on a flat hone. The knife should be stropped on one side until only a trifling unevenness is seen under the microscope, then an approximately equal length of time on the other. Wear on the blade is very slow and the machine may safely be left running overnight. When the edge is absolutely straight, the knife is run alternately on one side, then on the other, for a few seconds each during about a minute to remove the burr. A final polish is given by stropping for a minute to remove the burr. A final polish is given by hand on a soft strop free from dust or abrasive. The resulting edge is as perfect as can be produced.

The machine may be mounted on the wheels of a toy wagon for easy transportation.

Summary.—A simple automatic sharpener for microtome knives is described, which employs a revolving cylindrical fiber strip. The machine saves most of the labor of sharpening and produces a better edge than can be produced by hand. It is less expensive to build, more compact, less fragile and easier to use than machines now in use, and can be adapted to the largest knives.

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MORPHINE PENTOBARBITAL ANESTHESIA FOR DOG SURGERY*

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A NONVOLATILE anesthetic combination for dogs, which produces good surgical anesthesia and relaxation for a period of from one to two hours and assures postoperative recovery, is a distinct asset to the individual experimenter.

The short-acting barbiturates, pentobarbital and amytal, if used alone, give good anesthesia but recovery cannot be expected for from three to six hours. In addition, since these drugs are not analgesic, undesired restlessness and purposeless movement often characterize the recovery period.

We have been using in this laboratory a combination of morphine and pentobarbital sodium with gratifying results. The anesthetic dosage of pentobarbital sodium for the dog is from 0.025 to 0.035 gm. per kilogram intravenously. If morphine sulphate 0.01 gm. per kilogram and atropine sulphate 0.001 gm. per animal be given subcutaneously one and one-half hours prior to operation, the amount of pentobarbital sodium required is reduced to 0.005 to 0.015 mg. per kilogram, depending on the length of anesthesia required. Obviously, no fixed dosage can be predetermined for any given animal because of individual variability in reaction to the drugs.

When ready for operation, we inject intravenously a 1 per cent solution of sodium pentobarbital slowly, carefully observing respiration and other criteria of anesthesia, particularly the lid reflex. Slow injection (five minutes) permits of maximal control of depth of anesthesia with minimal dosage. The minimal amount of the drug required to produce anesthesia, if given in this manner, will usually last about one to one and one-half hours. Subsequent additions of this barbiturate by the same method of slow injection will extend the period of anesthesia if desired. Precaution against respiratory arrest must be taken by using slow intravenous injection of the drug on a symptomatic basis rather than by rapid administration of a fixed dose.

Recovery is gradual (one to two hours) and is free from excitement. Pharyngeal and laryngeal reflexes return early leaving no concern as to care or ultimate result from anesthetic procedure. We have found this combination highly satisfactory for any physiologic experiment in which the presence of morphine or a barbiturate is not specifically contraindicated.

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AN IMPROVED METHOD OF BLOOD CULTURE*

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THE following method of making blood cultures has given satisfactory results: A bottle, approximately 250 c.c. in size and containing 30 c.c. of dextrose phosphate broth, is fitted loosely with a two-hole rubber stopper bleeding attachment, the inlet tube of which should extend for about 6 cm. into the bottle, and which should have attached a No. 13 gauge needle, and

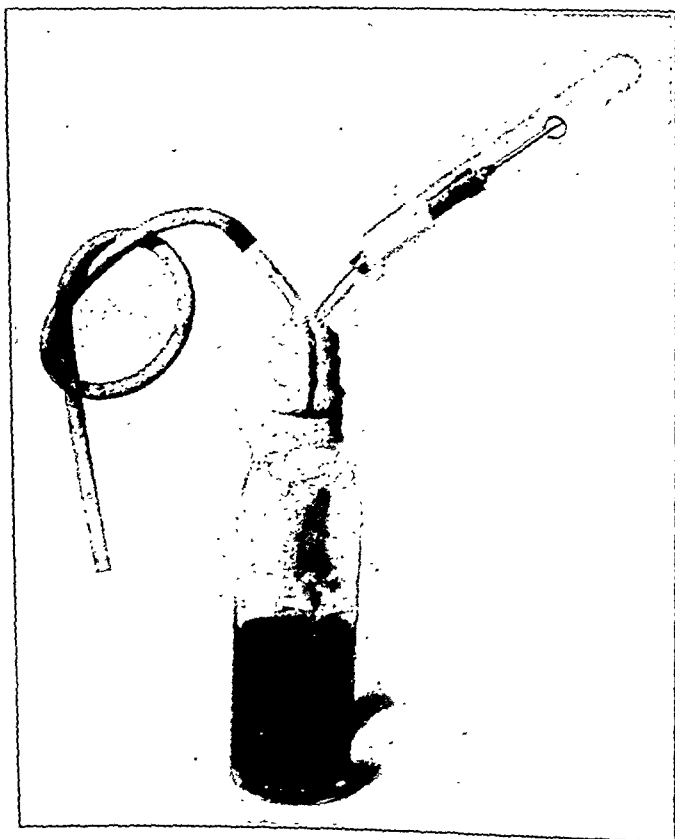


FIG. 1

is sterilized at a pressure of fifteen pounds for thirty minutes. Just before use, as recommended by Hadjopoulos and Burbank,¹ 10 c.c. sterile peptone solution, containing 2 drops of 5 per cent sterile sodium carbonate solution, are added. The rubber stopper is then fitted tightly and tied into position. The apparatus is now ready for use (Fig. 1). Into this bottle approximately 130 c.c. of blood is drawn from a suitable vein. The bottle is left to stand at room temperature for three hours in order to secure a firm adherent clot; it

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is now incubated at 37° C. and examined carefully each day for the growth of small pinpoint colonies on the edge of the clot or in the surrounding fluid. For identification of eventual growths, streaks on suitable mediums can be made. Using this method positive cultures were obtained in 43 of 67 cases (64 per cent), while the usual routine method yielded positive blood cultures only in 14 cases (21 per cent).

The advantages of the new method are (1) neutralization of natural complement or alexin of blood directly upon withdrawal by the peptone sodium carbonate solution¹; (2) direct transfer of blood to cultural medium with minimum handling and consequent lessened possibilities of contamination; (3) all gradients of oxygen tension necessary for the growth of different types of bacteria are provided; and (4) the ease of observance of growth.

REFERENCE

1. Hadjopoulos, L. G., and Burbank, R.: Alexin and Antialexic Bodies in Relation to Blood Culture Technic, *J. LAB. & CLIN. MED.* 15: 662, 1930.

THE PREPARATION AND STAINING OF FROZEN SECTIONS*

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FOR some considerable time there has been much discussion as to the relative value of the various methods used in the preparation of tissues for section and in the methods of sectioning the same. The Pathological Laboratory of the State Institute for the Study of Malignant Disease has for many years been one of the foremost champions of the frozen section method, and as we have many enquiries on this topic, it has seemed desirable to describe in some detail the actual technic in use in our laboratory with some discussion of results obtained and our reasons for the preference of this method.

During the past twenty years tissues from more than 125,000 surgical cases and from almost 1,000 autopsies have been sectioned by the frozen technic in the pathological laboratory of the Institute. Modifications in both the methods of cutting the section and in the technic of staining have been made from time to time, but our efforts have been primarily directed to the preparation of better sections rather than to the reduction of the time necessary to secure the same.

The frozen section method was at first advocated for its rapidity of execution. It was the only method practical for immediate diagnosis of suspicious tissue while a patient was held under anesthetic on the operating table, pending the report of the pathologist. Small portable microtomes were devised and these were especially designed for use in constricted quarters. With such equipment it was obviously difficult to obtain satisfactory or uniform sections. Where speed was the only objective, the quality of the sec-

*From the New York State Institute for the Study of Malignant Disease, Burton T. Simpson, M.D., Director.

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tion produced suffered; and this very fact caused many of the older and more experienced pathologists to condemn the method in its entirety.

While these objections were early realized in our laboratory, the desirability to the attending surgeon of prompt reports on biopsy material was also appreciated. Serious attempts were made to so modify and use the frozen section method that examinations could be promptly made without, in any way, sacrificing the quality of the section. At the present time we feel that our routine frozen sections are equal if not superior to sections produced by any other technic.

A prime necessity in the production of good sections is a suitable microtome. We have long since discarded the light, flimsy, portable type of microtome for a more solid instrument. At present we use a large sledge microtome, Spencer No. 860, which we have had modified so that we may use a freezing chamber. Sections produced on this rigid instrument are uniform in thickness

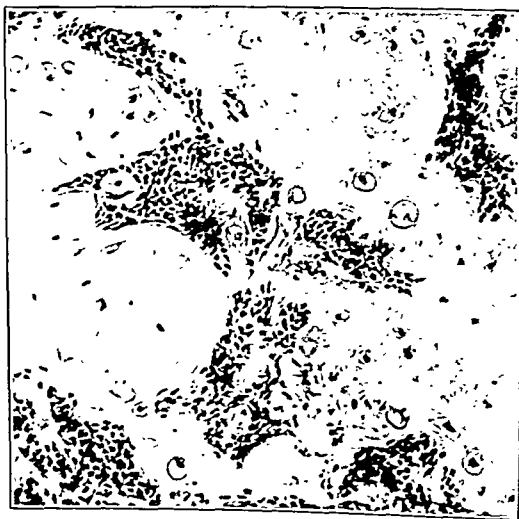


Fig. 1.—Photomicrograph made from a frozen section of a myxochondro-epithelioma. Stained with hematoxylin and eosin.

and can be consistently cut on properly fixed material at from $5\ \mu$ to $10\ \mu$. A sharp knife is, of course, essential. We employ 18 cm. and 25 cm. knives which are systematically examined and stropped each day before use. If any serious defect in the cutting edge is noted, the knife is laid aside for thorough sharpening.

FIXATION OF TISSUES

To secure good sections the tissue to be examined must be properly fixed. For the frozen technic, this is best accomplished by the use of 10 per cent formalin on thin slices of tissue. Where used, alcohol or other chemical fixative must be thoroughly removed by washing in water or other suitable solution. In the preparation of material for section the following steps are indicated:

1. Thorough fixation of the gross material preferably in 10 per cent formalin solution.

2. Careful inspection by the pathologist of such material with a view to the selection of suitable blocks for section.

3. Removal of blocks of tissue 1 cm. to 2 cm. square by 2 mm. to 3 mm. thick, and including if possible normal as well as pathologic portions of the tissue.

4. These blocks should be held in 10 per cent formalin until sectioned.

FREEZING OF TISSUES

For the freezing of the tissues carbonic oxide is used exclusively in our laboratory. We have found that tissues must be frozen to just the right consistency. If they are frozen too hard, the sections are apt to be brittle and to break up readily; if too soft the knife scrapes, rather than cuts the tissues and sections are not satisfactory. Various solutions of glucose, dextrin, gelatin, etc., have been used as embedding materials on the freezing stage, but we have

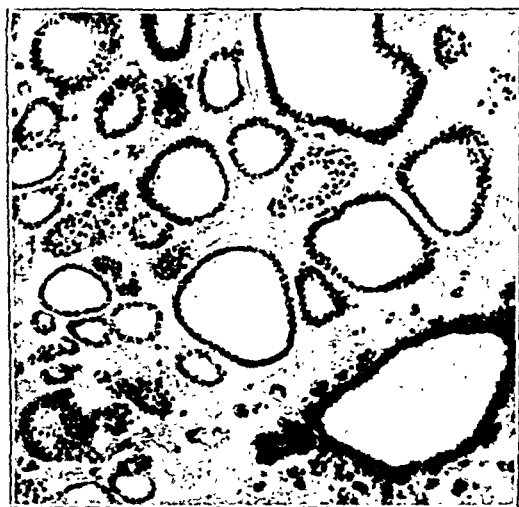


Fig. 2.—Adenoma of thyroid. Photomicrograph made from frozen section. Hematoxylin and eosin stain.

not found any of these to be of great practical value. Water in just sufficient quantity to fix the tissue firmly to the freezing stage has proved most satisfactory and any tissue too friable to give satisfactory sections in this medium alone should be immediately started through the paraffin or celloidin embedding process.

CUTTING OF THE SECTIONS

1. Insert a freshly stropped knife in the holder and systematically tighten all screws holding the knife and the freezing stage of the microtome. We have found that the best sections are secured when the knife is inclined at about 25° to the horizontal and when it sweeps over the block of tissue at an angle of approximately 30°

2. Set the microtome indicator at the proper point, 5 μ , 8 μ , 10 μ .

3. Rinse the block of tissue in distilled water and place in a small drop of water in proper position on the freezing stage.

4. Admit CO_2 to the freezing chamber in short blasts until the water and tissue *start* to freeze. Before the tissue is completely frozen the block is trimmed by bringing the knife over its surface, cutting away excess elevations and leveling the block.

5. When the tissue is well frozen, sections are secured in the usual manner by successive strokes of the knife.

6. Remove sections from the knife with the finger tip or with a wet camel's hair brush to a 10 per cent alcohol solution in a small Petri dish.

STAINING

Sections prepared as above are stained as follows:

1. Deliver sections into a flat Petri dish in the 10 per cent alcohol solution.

2. Transfer whole untorn sections into a shallow staining dish containing a solution of Harris hematoxylin, for from five to fifteen seconds.^{a, b}

a. Harris hematoxylin which we have found most satisfactory for routine staining is made up as follows:

Hematoxylin	5 grams.
Alcohol 95%	50 c.c.
Dissolve	
Ammonium Alum	100 grams.
Distilled Water	1,000 c.c.

Boil alum until dissolved: add hematoxylin. Bring whole to a boil. Then add 3 grams mercuric oxide (HgO) and cool *quickly*. The above is stock solution. For routine staining it should be further diluted as follows:

Hematoxylin (Stock)	300 c.c.
Glycerin	50 c.c.
Glacial acetic acid	12 c.c.

This stain darkens with age and is usually better a few days after preparation. When it becomes too dark, it is discarded and a fresh solution prepared from stock.

b. The placing of the staining dish over a window of the same diameter in the laboratory table and below which a 50 watt electric lamp is placed, gives a satisfactory illumination of the staining dish and allows sections to be handled more easily and more expeditiously.

3. Remove sections with straight section needle to large Petri dish containing fresh distilled water. Leave until all excess stain is washed out. Gentle agitation facilitates this process.

4. Transfer to a second Petri dish containing distilled water to which a few drops of NH_4OH have been added. In this solution the sections at once change from a purple to a blue color.

5. Sections are lifted, in rotation, into dishes containing 70 per cent, 85 per cent, and 95 per cent alcohols respectively, being left a few seconds in each.

6. Counterstain in third saturated solution of eosin in absolute alcohol for five seconds.

7. From the counterstain, sections are transferred for clearing to carbol xylol contained in a fairly large deep staining dish. From this solution they are mounted directly on the slide without the use of a section lifter.

8. Blot sections with filter paper and mount in balsam. This whole process of sectioning, staining and mounting can be carried out in less than five

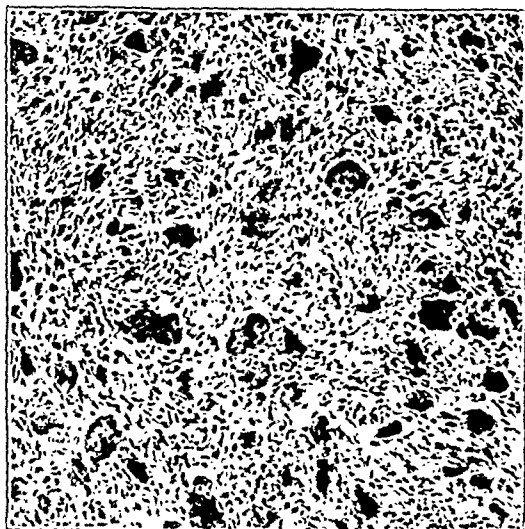


Fig. 3.—Giant cell tumor, epulis. Frozen section. Hematoxylin and eosin stain.



Fig. 4.—Squamous-cell carcinoma of skin. Photomicrograph made this year from frozen section made, stained and mounted in 1913, and in our files since that time. Staining by hematoxylin and eosin.

minutes. We have in some profusion tried out the various rapid methods using fresh unfixed tissues, polychrome stains, special embedding media, etc., but have not found any of them to give the same satisfaction as do fixed tissue sections stained with hematoxylin and eosin. The latter, moreover, give us permanent sections which retain their stain satisfactorily over long periods of time and which are of value in the permanent record of the case.

TABLE DIAGNOSIS

While in our laboratory we are of the opinion that the so-called "Table diagnosis" performed during operation is undoubtedly in many cases of great value to the surgeon, we feel that if this procedure is to be used, accuracy should be the primary consideration. Sufficient time should be allowed the pathologist to thoroughly examine the tissue in the gross and to select and properly fix the pieces of tissue for section. Our method in these cases is to fix small blocks, not more than 2 mm. thick or 1 cm. square, of selected tissue in strong formaldehyde solution which is kept at the boiling point. Fixation is usually satisfactorily accomplished in about fifteen seconds. We then proceed with the regular freezing and staining technic. Reports can usually be made on such tissues in four or five minutes from the time the tissue is handed to the pathologist. We feel strongly that the few additional moments necessary to allow the pathologist to make a careful survey of the tissue presented to him and to prepare and stain a section satisfactory for microscopic examination is, indeed, time well spent. If, on the other hand, the surgeon is unwilling to allow sufficient time for this type of examination, we are of the opinion that the whole procedure might better be abandoned.

Frozen sections when properly made and stained show on examination a brilliancy of staining which is attained by no other method of preparation. Due to a minimum subjection to chemicals, heat, etc., the cells retain their original size and shape to a much greater extent than is the case in paraffin or celloidin embedding. For the same reason also, sections prepared by the frozen technic are much more permanent than those prepared by any other method. Sections in our files for more than twenty years are today as well stained and as satisfactory for diagnosis as when first prepared. In fact, to some extent aging seems to improve these sections. During the past few years many photomicrographs have been made for the illustration of scientific articles from sections made routinely in the laboratory by frozen section technic. That this method is as satisfactory as any other for the purpose of photomicrography is demonstrated by several photomicrographs recently made from sections made during the usual laboratory routine. We also submit a photomicrograph of a section of a pearl forming squamous cell carcinoma which has been in our files for more than twenty years.

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A SIMPLE AUTOPSY STAND FOR MICE*

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UNDER the heading "Laboratory Aids" Dr. Raymond H. Goodale described in this JOURNAL (18: 422, 1933) a mouse holder made by nailing four spring-type clothespins to a piece of board. We find that such a holder is admirably suited for guinea pigs but for mice prefer one which is made from the cover of a Petri dish and four "Regal" paper clips. Two of these clips are

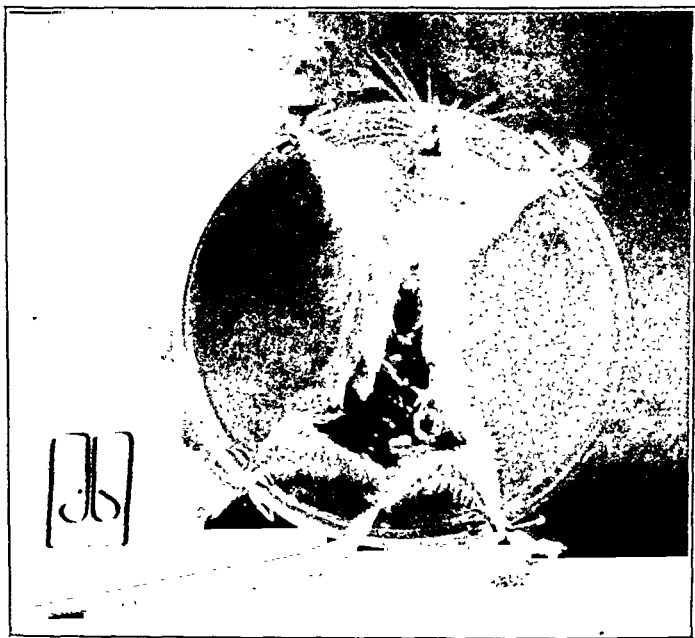


Fig. 1

slipped onto the rim on opposite sides of the dish, and the mouse's legs are drawn through the central slits of the clips. Fig. 1 shows the mouse in position and the type of paper clip which is used.

The advantages of this method are that the holder can be set up and disassembled in a moment; that infective material, such as washings from the peritoneal cavity, collect in the dish; and that after use the holder and mouse can be dropped into the ordinary basin of disinfecting fluid.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PNEUMOCOCCUS, Specific Precipitation Test for Standardization of Type I Antipneumococcus Serum, Brown, R. J. Immunol. 25: 149, 1933.

Forty-six Type I antipneumococcus sera were tested for precipitative activity with a solution of the Type I cellular carbohydrate. The results obtained agreed in 91.3 per cent of the cases with the results obtained by mouse protection tests. Eight concentrated antipneumococcus sera were similarly tested and found to agree in seven instances. The results indicate that the precipitation test described provides a titration which appears to be a reliable index of the development of the protective activity in the course of immunization; in the preliminary evaluation of the serum it can thus replace the more complicated protection test which, at present, however, should still be used in the final standardization.

SALMONELLA SUIPESTIFER, Infections in Man. Kuttner, A. G., and Zepp, H. D. J. A. M. A. 101: 269, 1933.

Four more cases of *Salmonella suipestifer* infection in man are reported: One in an adult and three in children. The adult developed a suipestifer septicemia and cystitis, following a radical antrum operation. Two of the children had arthritis and one of them showed osteomyelitis. The other child developed a suipestifer septicemia accompanied by severe purpura, during the course of bronchopneumonia. No complications such as arthritis, osteomyelitis, cystitis and purpura were observed in the group of cases reported by the authors in 1932. It is of interest that this organism, *Salmonella suipestifer*, Group II, can produce arthritis and osteomyelitis unaccompanied by fever.

Purpura has been observed as a rare complication in paratyphoid B fever. In none of the cases of suipestifer infection in man described in the literature has severe purpura with complete disappearance of platelets been described such as occurred in Case 2. Since purpura is a common symptom of pigs infected with the hog cholera virus, it was thought possible, in view of the interstitial bronchopneumonia found at autopsy, that in this patient *Salmonella suipestifer* might have been associated with the virus of hog cholera. Unfortunately, no experiments to determine the presence of the virus were undertaken. On the other hand, it is entirely possible that the purpuric manifestations were merely the result of a severe infection in a debilitated child and that some virus other than that of hog cholera was responsible for the pathologic picture in the lung. None of the other cases showed any evidence of purpura, and their platelet counts were entirely normal.

Three of the patients live in Baltimore; one lives in the country. In no instance was any association with infected pigs established, and none of the cases had any contact with each other. There was no history of a similar illness in any other member of the family, although the mother of one of the patients agglutinated the organism isolated from her daughter.

The organisms isolated from these four cases all belong to Group II, *Salmonella suipestifer*, although Group I is generally considered the American type, most commonly associated with hog cholera in this country. In recent years, however, Group II has been associated with increasing frequency from infected pigs in the United States.

URINE: Practical Method for Simultaneous Demonstration of Lactose and Glucose in, Kleiner, I. S., and Tauber, H. J. Biol. Chem. 100: 749, 1933.

To 1 c.c. of urine in a graduated 20 c.c. cylinder, 1 c.c. of a 4 per cent solution of copper sulphate is added, and then enough barium hydroxide solution to make the fluid faintly alkaline to litmus (approximately P_H 7.5 to 8); 5 c.c. is usually sufficient. Enough

water is added to make a total volume of 20 c.c. and the solution is mixed and filtered. If a faint opalescence persists, the results will not be affected and the trace of barium present need not be removed for either of the two determinations to follow. Two cubic centimeters of this filtrate is pipetted into a Folin-Wu sugar tube. Two similar tubes are prepared for standards, one of which contains 0.2 mg. of dextrose in 2 c.c. and the other 0.4 mg. of dextrose in 2 c.c. To each of the three tubes 2 c.c. of monose reagent is added and the tubes are heated in boiling water for eight minutes and cooled for two minutes. This reagent consists of a 1 per cent solution of barium hydroxide, to which is added 5 drops of a 1 per cent solution of sodium bisulphite for each 25 c.c. of reagent just before it is used. Another set is run with the Benedict alkaline copper reagent simultaneously, with the same amounts of filtrate, standards and reagent, boiling for six minutes and cooling for two minutes. To each of the tubes in both sets 2 c.c. of Benedict's color reagent is added and mixed with the contents. The tubes are allowed to stand for two minutes, water is added to the 25 c.c. mark, the contents are mixed thoroughly and colorimetric comparisons are made. If the color is too dark, the determination must be repeated with diluted urine for the copper-barium precipitation. With this procedure the monose method yields dextrose values that correspond closely with values obtained by the Benedict method.

BLOOD, Iron in Human. Sachs, A., Levine, V. E., and Apelsis, A. *Arch. Int. Med.* 52: 366, 1933.

The average iron content of whole blood, based on analyses of samples from 100 normal men, is 50.01 ± 2.56 mg. per hundred cubic centimeters. The average for 50 normal women is 42.67 ± 2.13 mg. per hundred cubic centimeters. The blood of women has a definitely lower iron content than that of men.

Iron in whole blood is almost entirely linked with the hemoglobin molecule. Since the quantity of nonhemoglobinous iron present in the serum and the cellular elements is very small, the error in calculating hemoglobin from whole blood iron is negligible.

On the basis that hemoglobin contains 0.335 per cent of iron we conclude that the average normal blood of men contains 14.93 ± 0.76 gm. of hemoglobin per hundred cubic centimeters, while the average normal blood of women contains 12.74 ± 0.66 gm. per hundred cubic centimeters.

By dividing the number of milligrams of iron per hundred cubic centimeters of blood by the first three figures of the red blood cell count, a quotient, which Murphy, Lynch and Howard designated as the iron index, is obtained. We have found the iron index for normal men to be 10.01 ± 0.65 gm., and for normal women, 9.6 ± 0.56 gm. of hemoglobin per hundred cubic centimeters.

The iron index is preferable to the color index because of the greater accuracy with which the former may be determined.

The iron color index, based on the ratio of the percentage of iron to the percentage of red blood cells, is also preferable to the older hemoglobin color index. The advantages to be derived from the clinical use of this index lie in its accuracy and in the retention of the convenient +1 or -1 designation of the older hemoglobin color index.

The red and white cell counts, iron, hemoglobin, iron index and iron color index in 63 clinical cases are also included in this report.

TUBERCULOSIS, Comparison of Blood Sedimentation Rate and Vernes Flocculation Test in Pulmonary, James, H. M. *Med. J. Australia* 11: 265, 1933.

The work of previous writers was confirmed in that both the blood sedimentation rate determination and the Vernes flocculation test are of considerable value in the control of treatment and prognosis in cases of pulmonary tuberculosis.

Providing all precautions are taken, and with certain recognized exceptions, the figures for both tests were comparable, particularly in those infections which were comparatively early or moderately advanced only. In the very advanced cases it was felt that the Vernes test represented the clinical condition less accurately than the blood sedimentation rate.

Of the two methods of investigation, it must be considered that the blood sedimentation rate estimation is more applicable to everyday clinical work and that there is no information given by the Vernes test which cannot equally be supplied by the blood sedimentation rate.

The advantages of the blood sedimentation rate method may be summarized as follows:

1. Expense: The Vernes test, because of elaborate laboratory methods and material, is a much more expensive test than the sedimentation test.

2. Simplicity: Any medical practitioner can do a blood sedimentation test at the bedside if necessary. Results are immediate and no calculations are necessary. With the Vernes test a certain amount of calculation is necessary, the human element becoming a factor to be considered.

3. Information: The blood sedimentation rate actually gives considerably more information than the Vernes test. In the Vernes test we have an end figure only. With blood sedimentation the rate of fall of the erythrocytes is plotted every five minutes and there is nothing in the Vernes test to correspond to the information received from a study of the curve. The difference between a diagonal and a vertical curve in the sedimentation test is often of paramount importance.

4. Comfort: The one cubic centimeter technic for the blood sedimentation is a very simple matter for the patient and occasions practically no discomfort. For the Vernes test it is necessary to withdraw at least ten cubic centimeters of blood. For the Vernes test also a fast of about fifteen hours is necessary before the test is made.

5. Transport: Blood for the Vernes test must be sent to a center where there are the requisite facilities. The blood sedimentation test may be performed anywhere.

In all the author feels that the blood sedimentation test is of equal value to the Vernes test and is not subject to the same number of fallacies.

Because of its ease of application and greater information supplied, it must be preferred to the more complicated Vernes test.

AGRANULOCYTOSIS, In Childhood, Givan, T. B., and Shapiro, B. Am. J. Dis. Child. 46: 550, 1933.

Twenty-nine cases of agranulocytosis in childhood are reviewed, and an additional case is added.

The instances of agranulocytosis in children with severe antecedent infection must be regarded as a unit.

Finally, a case is presented in detail in which antecedent infection was marked and agranulocytosis was superimposed. Adenine sulphate was used to stimulate the marrow centers, with good results, for with the reappearance of the granular elements, the peripheral blood stream, from which *Staphylococcus aureus hemolyticus* had previously been recovered, became sterile. Of note is the fact that when transfusions were started there was a coincident drop in the total number of white cells and in the percentage of granular cells. One injection of adenine sulphate, however, was sufficient to cause a return of the cellular elements to their previously attained level. The patient was then able to withstand two radical operations on the jaw, and recovery was uneventful.

TRICHOPHYTIN TEST, Report of 350 Cases, Muskatblit, E., and Director, W. Arch. Dermat. & Syph. 27: 739, 1933.

Three hundred and fifty patients were tested intradermally, of whom 300 were clinically mycotic and 50 were not mycotic.

Nonspecific reactions were observed in 4 per cent of nonmycotic cases.

The trichophytin test gave a positive reaction in 72.3 per cent of cases proved mycotic by the laboratory.

Clinical diagnosis was more frequently corroborated by the test than by the laboratory examination; for example, in clinically mycotic cases the test was positive in 60.3 per cent, while laboratory examination was positive in only 39.7 per cent.

Patients with highly inflammatory lesions gave a higher percentage of positive tests; however a positive reaction was found in many cases with limited and slightly inflammatory lesions.

The test was positive in cases in which the infection was due to various fungi of human and animal types, particularly in those caused by *Epidermophyton interdigitale*.

Monilia infections of the skin also gave a positive reaction but less frequently than cases due to filamentous fungi (*Trichophyton*, *Epidermophyton*, and *Microsporon*).

The intradermal test with fungous extracts, while not absolutely specific, is of considerable value in the diagnosis of fungous infections of the skin due to filamentous as well as to yeastlike fungi.

Common fungi such as *Trichophyton violaceum*, *Microsporon audouini* and *Epidermophyton interdigitale* yield an efficacious preparation when a mixed polyvalent extract is made of freshly isolated strains.

General allergic hypersensitiveness of the skin develops not only in deep inflammatory mycoses but also in many cases of superficial type.

Epidermophyton interdigitale, the most common causative agent of dermatophytosis in the country, is apt to cause a considerable degree of cutaneous allergy.

TULAREMIA: Culture Medium for Rapid Growth of *P. Tularensis*, Foshay, L. Am. J. Clin. Path. 3: 379, 1933.

Twenty-four grams of "Difco" dehydrated brain-veal agar and 2 gm. of nutrose are dissolved with gentle heat in one liter of distilled water containing 5 gm. NaCl, 0.3 gm. Na_2HPO_4 , 0.2 gm. KCl and 0.1 gm. CaCl_2 . The mixture is divided equally among four 500 c.c. flasks and set in the refrigerator to solidify. After solidification 0.1 gm. of cystine is placed in a small pile on the center of each surface. If care is taken not to stir the cystine around, the medium may be autoclaved safely at fifteen pounds for fifteen minutes without impairing the growth-promoting property of the cystine. Upon removal from the autoclave the flasks are set in a row and each is rotated vigorously in turn until the sedimented cystine is dissolved. This usually requires about ten minutes.

If only 250 c.c. of enriched medium is desired, three flasks are capped and put in cold storage and one is cooled to about 50° C. To this flask is added 10 c.c. of 25 per cent dextrose and 30 c.c. of either ascitic fluid or any sterile serum diluted 1:5. Other flasks are melted and enriched similarly as needed. Slanted tubes are capped as soon as set to prevent water loss. If a liter of finished medium is wanted the base medium may be solidified in one large flask, 0.35 gm. of cystine added, autoclaved, cooled and enriched with 40 c.c. of 25 per cent dextrose and 120 c.c. of ascitic fluid or diluted serum.

P. tularensis will grow rapidly and luxuriantly on the surface but not in the water of syneresis. Growth on slants is best at partial oxygen tension and, because of the tendency of the slants to slump when warmed, they are incubated in a slanting position. Partial oxygen tension is obtained by the tandem arrangement devised by Wherry and Oliver.

The medium is not good for preservation. Cultures will usually not remain viable, as judged by subculturing, for more than eight days. Strains that are being carried along on it are best transferred twice a week. For isolation of *P. tularensis* from infected rodent tissues it is only fairly good, inferior to blood-cystine-dextrose agar and to coagulated egg yolk. Its chief virtues are its capacity to promote rapid and abundant growth, and the short time and ease of making. The base medium can be stored indefinitely at low temperature, and tubes of the enriched medium are good for at least three months if water loss is prevented.

PENTOSURIA, Studies In, Enklewitz, M., and Lasker, M. Am. J. M. Sc. 186: 539, 1933.

Twelve cases of pentosuria are described, the sugar in all cases being 1-xyloketose.

All cases of "glycosuria" in which the concentration of reducing bodies in the urine remains unalterably below 1 per cent should be regarded as possible pentosurias and should be further investigated.

By examining urinary specimens voided before and after the ingestion of a liberal carbohydrate meal, it is often possible to differentiate cases of mild diabetes mellitus, renal diabetes and pentosuria. Fermentation tests, and testing with Bial's reagent will give additional valuable information as to the nature of the urinary sugar.

Xyloketose reduces alkaline copper solution in the cold.

The crystalline structure of racemic xylosazone is described.

The ingestion of amidopyrin markedly increases the output of urinary pentose.

Pentose excretion is uninfluenced by modification of the carbohydrate or protein content of the diet. It is also unchanged by rest, exercise or thyroid extract administered by mouth.

When fed 5 gm. of xyloketose a pentosuric excreted but 0.5 gm. more than usual. The urine of a control taking a similar amount of xyloketose showed no reducing substance. It appears that both pentosurics and normals can utilize or destroy xyloketose when it is administered by mouth.

SEDIMENTATION TEST, In Dermatology, Tulipan, L., and Director, W. Arch. Dermat. & Syph. 27: 759, 1933.

The red cell sedimentation test was performed in one hundred and fifteen patients with conditions representing thirty-five diseases of the skin. The method of Westergren was employed.

The sedimentation rate was usually normal in lupus erythematosus and in erythema multiforme, including the bullous variety.

It was usually increased in tuberculosis of the skin.

It was invariably increased in erythema nodosum and markedly so in the eruption due to phenolphthalein.

It was increased in dermatitis herpetiformis, whereas in other generalized grouped vesicular dermatoses, including dermatophytids, it was normal.

The sedimentation rate in epithelioma varied from normal to a pathologic increase, depending on the extent of the lesion.

It was high in a case of Hodgkin's disease in which there were cutaneous lesions.

It gave borderline readings in mycosis fungoides and erythroderma.

Normal readings were obtained in some of the more common dermatoses.

On account of the increased rate in six cases of pemphigus reported by Pessoa and Ribeira and in two cases reported here the sedimentation test is suggested as a possible aid in differentiating pemphigus from erythema multiforme bullosum.

BILIARY TRACT: Diagnostic Methods and Metabolic Studies in Disease of, Twiss, J. R., and Killian, J. A. Am. J. M. Sc. 186: 418, 1932.

A routine of study for diseases of the biliary tract is presented with findings in 10 control individuals and 30 patients in whom a complete investigation showed no evidence of biliary tract disease.

This investigation has included: History, physical examination, cholecystogram, duodenal drainage, inspection of bile specimens, microscopic examination of biliary sediments, chemical analyses of blood and bile, icterus index determination, van den Bergh reactions and the bacteriologic study of bile.

The findings in both series of patients are presented as standards for comparison with findings in cases of suspected gallbladder or liver disease.

Results indicate that with a normally functioning biliary tract these tests should show:

- a. A satisfactory "visualization" of the gallbladder on roentgenographic study.
- b. The presence of 30 to 60 ml. of concentrated bile during nonsurgical biliary tract drainage.
- c. The absence of pathologic elements on microscopic examination of the biliary sediment in all specimens.
- d. Little or no cholesterol in the D₁ and D₂ bile.
- e. Bile acids of the concentrated bile fraction 4 to 7 times greater than that of the duodenal bile.

f. The absence of pathogenic organisms which occur repeatedly in bile obtained by sterile duodenal drainages.

g. A blood serum icterus index reading of 4 to 8, a negative direct van den Bergh reaction.

SCARLET FEVER, Heterologous, Trask, J. D., and Blake, F. G. J. A. M. A. 101: 753, 1933.

The authors have demonstrated, as others before them have done or suggested, that heterogeneity exists among the toxins derived from scarlatinal streptococci. However, the chief interest of this communication lies in the demonstration and study of a new scarlatinal toxin from the blood and pleural exudate of cases of scarlet fever which failed to give a therapeutic response to potent scarlatinal antitoxin.

They do not wish to imply that this is the usual cause of failure in the antitoxin treatment of the disease because they have insufficient data on the incidence of this variety of scarlet fever. However, it is significant that Dr. Wadsworth has already prepared a polyvalent antitoxin which does neutralize the new toxin and the standard Dick toxin.

As regards the clinical features of the disease in three cases, it should be noted that pneumonia developed within the first few days in each case. It is of special interest and, perhaps, not without therapeutic significance, that a large amount of toxin was found in the pleural exudate in one case. The authors believe this to be the first demonstration of scarlatinal toxin in such material. They conclude that:

1. Heterogeneity exists among the toxins found in the blood in scarlet fever.
2. Heterogeneity exists among the scarlatinal antitoxins naturally found in man.
3. Polyvalency is desirable in therapeutic scarlatinal antitoxin.

SCARLET FEVER, Effect of Tonsillectomy on the Development of Immunity to, as Shown by the Dick Test, Kereszturi, C., and Park, W. H. J. A. M. A. 101: 764, 1933.

From a study of 34 tonsillectomized children as compared to 31 controls it is concluded that from this limited number of cases, it might be said that tonsillectomy has certainly no marked effect on the susceptibility to scarlet fever within six months after it is done, as demonstrated by the changing of a positive Dick test into a negative one.

HYDATIDIFORM MOLE, Differentiation of Pregnancy and, Dabney, M. Y., Flinn, G. G., and Dabney, E. G. J. A. M. A. 101: 771, 1933.

In view of the emphasis recently laid upon the greatly increased concentration of anterior pituitary sex hormone in the urine in hydatidiform mole and chorionepithelioma, the utilization of this fact as a means of differential diagnosis in the present case report is of definite interest.

In a patient with clinical symptoms suggesting hydatidiform mole, together with a strongly positive Friedman test after the injection of as little as 0.4 c.c. of urine, a dead five and one-half months' fetus was finally expelled.

The case is of further interest from a physiologic standpoint because of the unusually large quantity of anterior pituitary sex hormone demonstrated in the urine shortly before abortion of a nonviable fetus. It is possible that abnormal excretion of this hormone regularly accompanies fetal death without complete detachment of the placenta or that the increased quantity of hormone itself brings about defective fetal development and miscarriage and should be an indication for emptying the uterus in pregnancy, when it is not a sign of hydatidiform mole or chorionepithelioma. If this laboratory finding had been taken as an indication for therapeutic abortion, the present patient, a very anemic woman, would have been spared over a week of bleeding.

Further quantitative hormone studies in cases of threatened abortion and dead fetus are desirable, and the authors suggest revision of existing laboratory criteria for the diagnosis of hydatidiform mole and chorionepithelioma.

LIVER FUNCTION: Galactose Tolerance as a Measure of, Roe, J. H., and Schwartzman, A. S. *Am. J. M. Sc.* 186: 425, 1933.

A study of the galactose tolerance of normal subjects and patients with representative types of liver disease has been made by determining the blood and urinary galactose concentration following the ingestion of galactose by mouth. Data are presented showing that following galactose ingestion, the blood galactose concentration is a better indication of liver function than the urinary galactose excretion since it is not influenced to the same extent by a variable renal threshold.

Blood galactose concentrations similar to those of normal subjects were obtained in cases of cirrhosis, metastatic carcinoma, congestive heart failure, toxic and chronic degenerative hepatitis. The urinary galactose excretion in these cases was essentially the same as observed in normal subjects. These studies indicate that the galactose tolerance liver function test may not be expected to give evidence of diagnostic value in chronic liver disease.

Blood galactose values higher than normal were observed in cases of acute catarrhal jaundice. A normal response was obtained in a case of cholelithiasis with marked jaundice. These findings indicate that the galactose tolerance liver function test may be of value in the differential diagnosis of jaundices of infectious and noninfectious origins.

Based upon these studies a new technic for the galactose tolerance liver-function test is proposed. In this test 1 gm. of galactose per kilo of body weight is given by mouth under fasting conditions and the galactose content of samples of blood collected at hourly intervals for three hours following ingestion is determined. Data are presented for the interpretation of findings obtained by this technic.

MONONUCLEOSIS, Infectious: A Diagnostic Test for, Bunnell, W. W. *Am. J. M. Sc.* 186: 346, 1933.

The presence of a rather high concentration of heterophil antibodies, demonstrable in the form of sheep cell agglutinins, has been described in the active stages of 4 cases of infectious mononucleosis. Since then tests for heterophil antibodies have been made in over 2,000 cases representing various clinical entities, including additional cases of infectious mononucleosis. The diagnostic value of the test has become increasingly evident.

The serum, from 5 to 8 c.c. of human blood obtained as for a Wassermann test, is utilized for the procedure. For sake of conformity in results it is important to use a standard type of agglutination tube. The author has found a tube 100 mm. in length and 10 mm. in diameter most satisfactory and has used it in all tests, as a slight effect upon the readings is noticed with varying sized tubes. The use of a 2 per cent suspension of sheep cells is likewise desirable, as a heavier suspension interferes with the agglutination phenomenon.

If the serum is inactivated properly and the procedure carried out as described, there should be no hemolysis in the tubes to confuse the readings. (In serum which contains bile the sheep cells are usually hemolyzed and the tests unsatisfactory.)

The author has employed the sheep cell agglutinin test in over 2,000 cases representing 76 clinical conditions. With the exception previously noted, namely, serum disease, he has been unable to demonstrate an appreciable increase of heterophil agglutinins for sheep cells in the sera above the normal dilution of 1 to 8. In 15 cases of infectious mononucleosis he has found a consistent increase in all cases. The titers, ranging from 1 to 64 to 1 to 4,096, apparently depended to a considerable extent upon the stage of the disease at which the serum was obtained and upon the severity of the illness. None of the common conditions manifesting a similar clinical picture such as acute adenitis, tuberculous or syphilitic adenitis, Hodgkin's disease, acute or chronic lymphatic or myelogenous leucemia, aplastic anemia, purpura hemorrhagica, agranulocytic angina or Vincent's angina, has shown an increase in heterophil agglutinin titer.

In view of these findings it seems justifiable to accept the test for heterophil agglutinins for sheep cells as a valuable diagnostic procedure in differentiating infectious mononucleosis from a number of clinical conditions of a far more serious nature.

Cases presenting a suggestive clinical and blood picture whose blood serum shows an agglutination for sheep cells in a dilution of at least 1 to 64 can apparently safely be diagnosed as infectious mononucleosis, and a favorable prognosis given.

PELLAGRA: Review of Cases With Special Reference to Gastric Secretions, Mulholland, H. B., and King, R. L. *J. A. M. A.* 101: 576, 1933.

The results of gastric analyses in 107 typical endemic cases of pellagra admitted to the University of Virginia Hospital during the past eighteen years confirm the results found by previous observers in regard to the common occurrence of achlorhydria in this disease, even after due allowance has been made for the presence of this condition in normal persons of different ages. The examination of some patients indicated that this condition might be more or less permanent (true anacidity), but in a few instances some degree of normal function returned. The assumption, however, that the presence of achlorhydria was necessarily correlated with such other manifestations as stomatitis, diarrhea, involvement of the central nervous system and anemia was not borne out by this study. There is no implication that achlorhydria is a primary or uniformly antecedent manifestation of this deficiency disease syndrome.

TUMORS, Carcinoid of the Gastrointestinal Tract, Raiford, T. S. *Am. J. Cancer* 18: 803, 1933.

Twenty-nine cases of carcinoid tumors in the gastrointestinal tract have been studied with regard to their clinical and pathological significance. Six of these were malignant and had metastasized to the regional nodes of the liver.

The origin of carcinoids has been a subject of much controversy. It is now generally conceded that they arise from the cells of Kultschitzky or the argentaffine cells occurring in the normal intestinal mucosa. The origin and function of these cells remain a matter of speculation, but they are thought to be related in some way to the chromaffin system.

The pathology of the group is characteristic. Occurring most commonly in the appendix, the carcinoids are yellow, submucous tumors which encroach upon the lumen. Next in frequency in the small intestine, they form small submucous or pedunculated nodules which are usually asymptomatic and are not recognized clinically. Rarely found in the stomach and large intestine, they are of more significance. They are larger, and metastasis occurs in a greater number of cases. They are clinically not unlike adenocarcinomas save for general symptoms of less severity.

The histology of the carcinoids is typified by groups and columns of cells surrounded by a dense stroma of hypertrophied connective tissue and smooth muscle. The cells are small and regular in size, the granular cytoplasm having an affinity for silver. The nuclei are uniform and are heavily dotted with chromatin particles.

The prognosis of the group as a whole is good. Only in the minority of cases which have metastasized is the outlook grave. Even in such an event, if the tumor is recognized clinically before metastasis has become widespread, a good result may be attained through surgical intervention.

MENINGOCOCCI, Variation in Agglutination of Stock Cultures of, Maegrath, B. G. *Brit. J. Exper. Med.* 14: 219, 1933.

Great variation in agglutination by "type" sera occurs among single colonies of stock meningococci.

Littledale (Type I) strains show a tendency to overlap into Type III to such an extent that these types appear to be approaching interchangeability.

There is no evidence of a division of group and specific antigens among single colonies derived from stock type cultures.

The so-called Types I and III of the meningococcus are in all probability not fixed types.

MENINGOCOCCI, Rough and Smooth Variants in Stock Cultures of, Maegrath, B. G. *Brit. J. Exper. Med.* 14: 227, 1933.

Certain variants of stock strains of meningococci can be detected by the characteristics of their growth on homologous immune-serum agar.

One variant develops haloes in this medium and the other does not.

Investigation of the properties of these variants has indicated that they are of "smooth" and "rough" nature respectively. This is supported by differences in growth in various media, in their fermentation and precipitin reactions and in their effects on guinea pigs.

Agglutination and absorption reactions with sera prepared from the variants do not agree with the results obtained by Enders with his "S" and "R" forms.

These variants are being further investigated.

MENINGITIS, Lactic Acid of Spinal Fluid in, De Sanctis, A. G.; Killian, J. A., and Garcia, T. Am. J. Dis. Child. 46: 239, 1933.

Lactic acid is a product of the inflammatory process, and in meningitis the lactic acid of the spinal fluid exceeds that of the blood, and is independent of it. The authors believe that variations in lactic acid of spinal fluid offer more dependable information concerning the progress of a case of meningitis than any other laboratory procedure at our disposal. Its practical use is further enhanced by the fact that within the last few years the technic of its determination has been greatly simplified and its accuracy increased.

SILICOSIS, Silicon Dioxide Content of Lungs in Health and Disease, Mc Nally, W. D. J. A. M. A. 101: 584, 1933.

From experimental observations it is concluded that the normal lung contains 1.13 mg. of silicon dioxide per gram of dried tissue.

A chemical examination of the lungs should be made in every case coming to autopsy, in which there is a history of a dusty occupation.

Any lung containing over 2 mg. of silicon dioxide per gram of dried tissue indicates undue exposure to a dusty atmosphere.

LIVER FUNCTION: Urinary Test as Index of Absorption of Gall Bladder Dye When Given Orally, Rudisill, H., and Hemingway, W. W. J. A. M. A. 101: 593, 1933.

The test, a modification of the Kendall test, has for its purpose the detection of gallbladder dye in the urine after oral administration.

To about 50 c.c. of urine from 1 to 2 gm. of stick sodium hydroxide is added. The mixture is heated moderately, to prevent spattering, in an evaporating dish. When evaporation is nearly complete, exactly 1 gm. of powdered potassium nitrate is sprinkled over the extract, and this mixture is fused at moderate heat until the carbon has all disappeared and the "melt" appears white. When the "melt" has cooled, 20 c.c. of distilled water is added, in which the "melt" readily dissolves. The solution is then filtered and the filtrate is acidified with dilute sulphuric acid until blue litmus is turned red. About 0.5 gm. of sodium nitrite is then added to the filtrate and the mixture is placed in a separatory flask. Five cubic centimeters of chloroform is added and the mixture shaken vigorously. A pink color in the chloroform layer indicates the presence of iodine.

Three arbitrary standards of absorption are described:

1. Good Absorption: This is indicated by a deep purplish pink and is the color obtained as a routine in the normal gallbladder visualization. This color can be reproduced by dissolving 5 mg. of resublimed iodine in 100 c.c. of chloroform.

2. Moderate Absorption: This is indicated by an ordinary pink and can be simulated by 2.5 mg. of resublimed iodine in 100 c.c. of chloroform. This degree has been obtained even with excellent visualization of the gallbladder in patients with poor kidney function.

3. Poor Absorption: This gives a very faint pink that is best seen against a white background, and the standard is made by dissolving 1.25 mg. of resublimed iodine in 100 c.c. of chloroform. When the absorption is poor, the examination should be repeated unless there is normal visualization of the gallbladder.

So far the only source of error encountered has been an erroneous index of the degree of absorption in patients with poor kidney function, as mentioned.

In case of nonvisualization of the gallbladder after the dye has been given by mouth, if the described test is positive there is no question of nonabsorption of the dye, and the gallbladder, liver, or duct system must be abnormal.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Discovering Ourselves—A View of the Human Mind and How It Works*

THIS is a volume on normal and abnormal psychology written for the patient. Many such volumes have appeared in the past, most of which have been in greater or less measure infiltrated with ballyhoo. No one but a psychiatrist extremely well versed in human behavior and psychic reactions to suggestion would be qualified to write a popular volume that could be relied upon not to do almost as much harm as good. The problem is still further complicated by the need for a writer who can omit scientific verbiage, and discourse in a manner that will be intelligible to and will continue to hold the interest of the average intelligent layman.

Strecker and Appel have succeeded admirably in fulfilling these requisites. If you have been called an introvert and you want to know what it is you can find a straightforward explanation in this volume, one that will not increase your tendency to introversion but it will enable you to combat logically any morbid exaggerations that you might have in this direction. The extrovert and others with borderline psychiatric states can, indeed, understand themselves after a perusal of this volume.

It is one that can safely be recommended to the intelligent layman.

Histopathology of the Peripheral and Central Nervous Systems†

IT CANNOT be disputed that, while neuropsychiatry is truly a specialty, and an exacting one, neuropsychiatric problems are very often first encountered, although not always recognized as such, in general medical practice. Nor can it be easily gainsaid that for an understanding of the clinical phenomena in this field some grasp of neuropathology is essential.

For these reasons, and because, further, adequate discussions of neuropathology from the viewpoint of the physician at large have not been numerous, the present volume is presented.

It is divided into Part I (four chapters, 56 pages), covering diseases of the peripheral nerves; Part II (seven chapters, 131 pages), in which diseases of the spinal cord are discussed; Part III (nine chapters, 253 pages), covering diseases of the brain; and Part IV (seven chapters, 30 pages), devoted to staining methods used by the author.

It is apparent from these data that the volume is not encyclopedic, nor was it intended to be. On the contrary, there is presented a clear, well-written succinct and yet satisfactorily comprehensive survey of neuropathology which while of interest to workers in this field, should be gratefully received not only by the physician at large but also by the general pathologist. To these it should be very welcome and from them it will doubtless be accorded a hearty reception.

The illustrations, with but few exceptions, are well chosen, excellently reproduced, and informative. The format is acceptable.

This book is well fitted for a place on the doctor's shelf of reference texts.

*Discovering Ourselves. A View of the Human Mind and How It Works. By Edward A. Strecker, A.M., M.D., and Kenneth E. Appel, Ph.D., M.D. Cloth, pages 306. The Macmillan Company, New York, 1931.

†Histopathology of the Peripheral and Central Nervous Systems. By George B. Hassin, Professor of Neurology, University of Illinois. Cloth, pp. 491, 229 figures. William Wood & Co., Baltimore.

Modern Aspects of Gastroenterology*

THE purpose of this book is to present in an orderly and systematic manner the most valuable of the newer methods of investigation, diagnosis, and treatment in the field of gastroenterology.

While the viewpoint of the author naturally reflects European studies, American literature has not been neglected.

Gastrointestinal, hepatic, and pancreatic diseases are all considered in a satisfactory manner. The price of the volume seems a little high but is probably accounted for in part by the number of illustrations.

Filterable Virus Diseases in Man†

WHILE small, this volume presents a very comprehensive yet succinct survey of the present state of knowledge concerning those diseases at present regarded as caused by filterable viruses.

For those desiring a bird's-eye view of a subject still under investigation and in a state of flux, the book may be accepted as an excellent summary of the investigations in this field to date.

Manual of Veterinary Bacteriology‡

THOSE already familiar with this excellent book will not be surprised to find that it has reached a second edition. To those to whom it comes as a new book, it will be found an authoritative and comprehensive reference work of value, not only to the veterinary, but to the laboratory worker at large. Indeed, considering the number and importance of the diseases which may be transferred from animals to man, this volume may well be read by the physician with profit.

It can be highly recommended as an excellent and standard reference text.

*Modern Aspects of Gastroenterology. By M. A. Arafa, Medical Assistant to Guy's Hospital, London. Cloth, pp. 374, 32 plates, William Wood & Co., Baltimore.

†Filterable Virus Diseases in Man. By Joseph Fine, M.D., Assistant to the Professor of Public Health, Edinburgh, University. Cloth, pp. 144, William Wood & Co., Baltimore.

‡Manual of Veterinary Bacteriology. By Kelsor R. A., Major, Veterinary Corps, U. S. Army. Cloth, pp. 552, 93 figures. The Williams & Wilkins Co., Baltimore.

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EDITORIAL

Hyperinsulinism

ALTHOUGH it has been about twenty years since the development of reliable clinical methods for blood sugar determination, the recognition of the etiology of occasional abnormally low blood sugars has been a development of the postinsulin era. Hypoglycemia due to hyperinsulinism is often but not invariably a constant laboratory finding in the individual case. It is accompanied by certain symptoms which we are accustomed to consider as rather neurotic; nervousness, periods of weakness, abnormal sweating, tachycardia, sinking spells, and occasionally even unconsciousness. One has the feeling that if blood sugar determinations were made more frequently and more carefully on the hyposthenic nervous individual with symptoms such as these, grades of hyperinsulinism would be recognized more frequently. Such a discovery would be a great boon to this large group of sufferers. Unfortunately, however, this has not so far been a very common finding even in the type of individual men-

tioned. John¹ remarks on the apparent relative rareness so far of true hyperinsulinism stating that no one physician sees a score of such patients a year. At the same time it is highly desirable to recognize those cases which one does see.

The logical procedure in the treatment of individuals with symptoms associated with abnormally low blood sugar would appear to be the administration of sugar. This is the procedure which is usually followed, although failures have been reported by this method, probably due to the fact that the feeding of large quantities of carbohydrates stimulates the insulogenic function, thereby creating a vicious circle. The next therapeutic development appears to have been rather more successful and consists in the restriction of carbohydrates to a comfortable minimum with the addition of a reasonable abundance of fats, designed to delay the entrance of carbohydrate into the circulation, thereby avoiding overstimulation of the islets.

Partial resection of the pancreas for hyperinsulinism is a possibility and might appear as logical as subtotal thyroidectomy were it not for the tremendous operative risk and the probability that the remaining tissue would undergo rapid hyperplasia, reproducing the original state. Such operations have been done but the end-results have not yet been observed.

Henry J. John reports an entirely new therapeutic procedure, one which at first glance probably appears quite illogical but which on more detailed study gives promise of being the most logical procedure recommended so far. This briefly is the administration of insulin in hyperinsulinism. He reports its use in only one case but in this case the results were almost spectacular.¹ The single case is reported, with the idea in mind that not many cases of hyperinsulinism are seen, and that it is well therefore to report the results so that others may try the procedure in their occasional cases.

In the particular case reported John places the threshold at which insulin output is stimulated, as 200 mg. per 100 c.c., blood sugar. If the patient's digestive glycemia exceeds 200 the pancreas is stimulated to pour out insulin. If the glycemic level can be kept below 200 the pancreas will not be stimulated. The procedure therefore consists in giving insulin (ten units in this case) *after* meals, and before the peak of the blood sugar curve has been reached. This exogenous insulin starts the curve on its downward trend before it has reached 200. In this way the pancreas is kept at rest. In spite of the fact that the patient already had a hyperinsulinism, the only single time at which she experienced hypoglycemic symptoms during the period of treatment was on one occasion when by mistake she took her insulin before her meal instead of after her meal.

The blood sugar level or threshold below which hypoglycemic symptoms appeared was 70. The administration of a standard meal containing 100 grams of carbohydrates, and without the use of exogenous insulin was followed by a digestion glycemia exceeding 200. This was followed by a fall to 40 mg. per 100 c.c., well below the threshold for hypoglycemic symptoms. The interpretation was that the digestion glycemia of 230 stimulated the pancreas which put out a superabundance of insulin with a resulting hypoglycemia, sufficient to produce symptoms.

On the same diet, with insulin administered after the meal, the peak of the curve was reached at about 160, not high enough to stimulate the pancreatic tissues, and there was no subsequent hypoglycemic trough and no reaction.

During the two months in which the patient was receiving insulin, ten units after meals, she remained entirely symptom-free and gained 17 pounds. The dosage was then gradually reduced and later discontinued. One month later, at the time of the report, clinical improvement had continued without interruption.

Functional hyperinsulinism may result either from overaction of the islands of Langerhans or from some defect in the nerve control mechanism. Organic hyperinsulinism may accompany carcinoma or adenoma of the islands. Neither carbohydrate feeding nor the feeding of fat and carbohydrate has been of benefit in hypoglycemia due to hyperinsulinism from adenoma of the island. John points out that in individuals with hyperinsulinism it is important to differentiate functional disease from neoplasm of the pancreas. Often this can only be finally done following laparotomy. He suggests that on theoretical considerations the response described in his case report would not occur in individuals with organic pathology in the pancreas. In such case, the therapeutic procedure described might be developed into a therapeutic test for differentiation between functional and organic hyperinsulinism.

REFERENCE

John, Henry J.: A Case of Hyperinsulinism Treated with Insulin. Preliminary Report. *Endocrinology* 17: 583, 1933.

—W. T. V.

To Stimulate Original Research Work on Goiter

The American Association for the Study of Goiter, for the fifth time, offers Three Hundred Dollars (\$300.00) as a first award, and two honorable mentions for the best essays based upon original research work on any phase of goiter presented at their annual meeting in Cleveland, Ohio, June 7th, 8th, and 9th, 1934. It is hoped this will stimulate valuable research work, especially in regard to the basic cause of goiter.

Competing manuscripts must be in English, and submitted to the Corresponding Secretary, J. R. Yung, M.D., 670 Cherry St., Terre Haute, Ind., U. S. A., not later than April 1, 1934. Manuscripts received after this date will be held for the next year or returned at the author's request.

The First Award of the Memphis, Tenn., 1933 meeting was given Anne B. Heyman, A.B., M.S., University of Michigan, Ann Arbor, Mich., "The Bacteriology of Goiter and the Production of Thyroid Hyperplasia in Rabbits on a Special Diet."

Honorable mentions were awarded J. Lerman, M.D., and W. T. Salter, M.D., Huntington Memorial Hospital, Boston, Mass., "The Calorigenic Action of Thyroid and Some of Its Active Constituents," Prof. Dr. Stefan Konsuloff, Sofia, Bulgaria, "Experimental Studies on Etiology of Goiter."

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CLINICAL AND EXPERIMENTAL

A STUDY OF THE GRANULAR AND FLOCCULAR TYPES OF AGGLUTINATION WITH *B. TYPHOSUS**†

RUTH GILBERT, M.D., MARION B. COLEMAN, B.S., AND ALICE B. LAVIANO, M.S.,
ALBANY, N. Y.

ALTHOUGH the agglutination test with *B. typhosus* is among the oldest and most universal of laboratory procedures for demonstrating evidence of infectious disease, a standardized technic which meets all needs has still to be devised. The inadequacies of the classical microscopic test are obvious. The use of living culture introduces a variable which can be controlled only within relatively wide limits. Moreover, recent investigation indicates that an analysis of certain of the agglutinative properties demonstrable with killed suspensions may furnish information of greater diagnostic significance than can be secured from the results of a single test with a living culture.

Two types of agglutinative properties in serum were demonstrated in 1903 by Smith and Reagh¹ while they were working with motile and nonmotile strains of the hog cholera bacillus. In performing agglutination tests with serum produced with the motile form, they observed a "fluffy precipitate" of the homologous strain and a "granular precipitate" of the nonmotile form which occurred only in relatively high concentrations of the serum, while both strains were agglutinated in a granular manner and to practically the same degree in serum produced with the nonmotile form. Hence, they concluded that the flagella stimulated the production of one type of agglutinative property and the body of the microorganism stimulated the production of another type. Weil

*From the Division of Laboratories and Research, New York State Department of Health.
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†Presented before the Society of American Bacteriologists, Baltimore, Maryland, December 30, 1931.

and Felix,² in 1917, reported differences in the character of agglutination obtained with *B. proteus* X 19 in sera from animals artificially immunized with this microorganism and sera from patients having typhus fever. Similarly, they were able to distinguish two types of agglutination obtained with cultures from "Hauch" and "ohne Hauch" colonies of *B. proteus* X 19; hence, they used the initials "H" and "O," respectively, for designating these two types of colony. These letters were later applied to the character of the agglutination obtained with the respective cultures and to properties in sera giving rise to the two forms of reaction. These types of clumping have also been designated as coarse and fine, and as large and small. The terms "floccular" and "granular" describe so well the character of the clumping and correspond so closely to the original terminology employed by Smith and Reagh¹ that their exclusive use seems desirable, not only for purposes of clarity and uniformity, but also in recognition of the priority of these authors' observations. The observations of Weil and Felix² and those of later investigators, notably Büchner and Zorn,³ indicate that the properties in sera which give rise to the granular type of agglutination are heat-labile, while those inducing floccular agglutination are relatively heat-stable. On the other hand, the corresponding properties in the bacteria are found to behave in an exactly opposite manner, the former being less sensitive to heat than the latter. Further studies, especially those of Weil and Felix^{4, 5} and of Weil, Felix and Mitzenmacher,⁶ demonstrated similar properties in typhoid bacilli and other microorganisms of enteric disease. Certain species, particularly *B. typhosus* and *B. enteritidis* Gärtner, were shown to have a common factor demonstrated by a granular type of agglutination, while the floccular agglutination was thought to indicate a species-specific reaction. Felix,⁷ in 1924, reported that the granular or group-agglutinative property may appear in the sera of typhoid fever patients earlier in the disease than the floccular or specific property and indicates a favorable prognosis. In this investigation, Felix employed living cultures, i.e., one strain of *B. typhosus* particularly rich in the group factor, a second showing both factors in a normal manner, and a strain of *B. enteritidis* Gärtner as a control for the granular type of agglutination.

Felix and Olitzki,⁸ in 1928, studied the characteristics of killed suspensions of *B. typhosus* and related microorganisms and reported that low concentrations of phenol and formalin definitely inhibited the granular but not the floccular type of agglutination. On the other hand, they found suspensions treated with alcohol by the method of Bien and Sontag⁹ satisfactory for demonstrating the former but not the latter type of agglutination. The observations of these workers indicate that a reaction with a formalin- or phenol-treated suspension of typhoid bacilli furnishes evidence that the patient has typhoid fever, that he has had such an illness in the past, or that he has been inoculated with typhoid vaccine, while a reaction with a suspension treated with alcohol indicates that the patient has an infection due either to the species of bacteria used in the test or to one which is allied to it. Burnet,¹⁰ in 1924, studying a limited number of cases, confirmed the observations of Felix with respect to the sera of typhoid fever patients and of persons who had received typhoid vaccine. Verzáz,¹¹ in 1924, reported the alcohol-treated suspensions of *B. typhosus* and related micro-

organisms to be more sensitive in agglutination tests than those treated with phenol. Gardner¹² and Whitehead¹³ agree in general with the findings of Felix and his coworkers, but believe that agglutination with the alcohol-treated antigen may often be demonstrated, in low dilutions at least, in the sera of persons who have received typhoid vaccine and in those of certain normal individuals as well. Felix¹⁴ reports that floccular agglutination does not occur in normal serum, whereas the granular type of agglutinative property may be demonstrated in dilutions as high as 1:100. Craigie,¹⁵ who made an exhaustive study of flagellar and somatic suspensions of the typhoid bacillus, confirms the conclusions of previous investigators that two types of agglutinative property exist, one acting on the flagella and the other on the somata. He also demonstrated very clearly that differences in the macroscopic appearance of the granular and floccular types of agglutination are associated with morphologic properties. A recent report by Eldering and Larkum,¹⁶ indicates that they have found the employment of two antigens of practical value in the examination of sera for evidence of typhoid fever.

For several years, we have hoped to be able to use killed microorganisms in the agglutination test for evidence of typhoid fever. In 1922, Gilbert and Moore¹⁷ reported failure to obtain satisfactory results with formalinized cultures of *B. typhosus* in microscopic agglutination tests. Subsequently, an extensive study of formalin-treated suspensions in macroscopic tests demonstrated that occasionally the sera from patients with typhoid fever which react in the test with living culture do not agglutinate such antigens. Most encouraging results, however, have been obtained in a series of macroscopic tests in which, in addition to a suspension of microorganisms killed with formalin, one treated with alcohol, as outlined by Bien,¹⁸ has been employed. A strain (No. 305) of *B. typhosus* isolated in October, 1929, from a specimen of feces received from a typhoid carrier was found to be suitable for the preparation of these antigens; a nonmotile variant has proved especially satisfactory for that treated with alcohol. Three other strains of *B. typhosus* which have been maintained for a number of years in the bacterial collection, No. 21 Bender, No. 62 Rawlins, and No. 176 Pfeiffer, were found unsuitable for the preparation of the alcohol-treated suspension.

Our experience has indicated that both types of antigen remain satisfactory for at least six months.

TECHNIC USED IN THE MACROSCOPIC AGGLUTINATION TEST

Before the antigens are prepared, the cultures are plated to insure the use of strains which have little, if any, tendency to develop "rough" colonies. Should such colonies be found, "smooth" colonies are fished and replated, a process which may need to be repeated several times. The culture for the formalin-treated antigen should be actively motile.

Formalin-Treated Antigen.—The twenty-four-hour growth from beef-in-fusion agar in a pint Blake bottle is suspended in from 20 to 30 c.c. of 0.85 per cent salt solution containing 0.2 per cent formalin. After seventy-two hours in the refrigerator, tests for bacterial growth are made and, if necessary, more

formalinized salt solution is added. After the microorganisms have been killed, the turbidity is adjusted to correspond to barium sulphate standard No. 3¹⁰ by the addition of salt solution containing 0.2 per cent formalin, and the agglutinability of the suspension is tested.

*Alcohol-Treated Antigen.*¹¹—The twenty-four-hour growth from beef-in-fusion agar in a pint Blake bottle is suspended in 10 c.c. of 0.85 per cent salt solution containing 0.5 per cent phenol. The growth from several bottles is combined and one-half the volume of absolute alcohol or a proportional amount of 95 per cent alcohol is added slowly, while the suspension is constantly stirred. It is then allowed to remain at a temperature of from 35 to 37° C. for about eighteen hours after which the supernatant fluid is decanted and tested for bacterial growth and agglutinability. After determination of the dilution necessary to secure a density equivalent to that of barium sulphate standard No. 3, sufficient alcohol is added to the concentrated suspension to give 2.5 per cent in the diluted antigen, which should contain not more than 0.04 per cent phenol.

Equal amounts (0.3 c.c.) of the suspensions, diluted to correspond in density with barium sulphate standard No. 3, and serum dilutions of 1 to 10, 20, 40, 80, and 160 are combined and incubated at from 50 to 55° C. for from eighteen to twenty hours. Clear supernatant fluid and, after gentle shaking, clumps which are definitely discernible to the unaided eye constitute a characteristic reaction. Vigorous shaking should be avoided since clumps of the floccular type are easily dispersed.

TECHNIC USED IN THE MICROSCOPIC AGGLUTINATION TEST

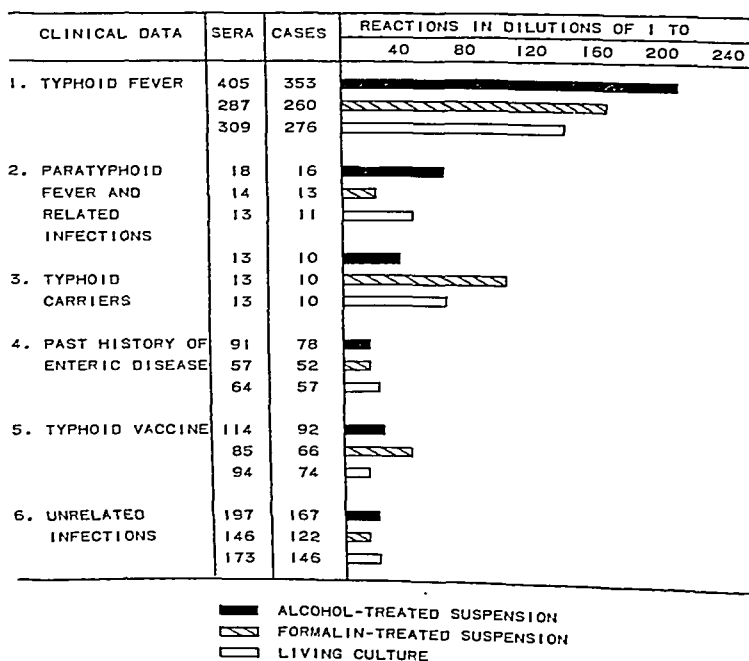
A two- to four-hour culture in bile peptone solution¹² is employed, the density of which is approximately one-half that of barium sulphate standard No. 1.¹⁰ One loopful of each of the serum dilutions used in the macroscopic test is combined with one loopful of culture on cover slips, which are inverted over vaseline-ringed hollow-ground slides. The tests are incubated for one hour at from 35 to 37° C. and the reactions read with a microscope, either a No. 6 or a No. 7 objective being used. Almost complete loss of motility and clumping of the majority of the microorganisms is regarded as characteristic agglutination.

It has been customary to consider of diagnostic significance definite agglutination reactions in the 1:40 dilution in the microscopic test and only those in the 1:80 or higher dilution in the macroscopic test.

Since this study was undertaken, approximately 4,000 sera have been examined. As the use of the various antigens was not begun simultaneously, the number of sera tested with each differs. The specimens have been divided into six groups, according to the clinical data which accompanied them. In the determination of the average agglutination titers, reactions in dilutions higher than 1:320 have not been considered. Only the granular type of agglutination has been observed with the alcohol-treated suspension. Usually the suspension treated with formalin has reacted in a floccular manner, but occasionally, especially in sera from patients having infections with organisms of the paratyphoid enteritidis group, granular clumping has also been noted.

In the first series, in Chart 1, which represents either clinical or bacteriologically proved cases of typhoid fever, the average titer with the alcohol-treated

antigen is appreciably higher than that with the other two. Of 287 sera tested with all three antigens, 259, or 90 per cent, gave reactions considered of diagnostic significance with the alcohol-treated suspension, and 252, or 87 per cent, with the living culture, while only 220, or 76 per cent, definitely agglutinated the formalin-treated antigen. Twenty-one gave no more than a partial reaction with any of the antigens, but of these, 9 were collected within the first week of illness, while in one instance the duration of illness could not be determined. Two of those giving little or no agglutination, which were collected during relapses from two to two and one-half months after the onset of illness, seem worthy of note. *B. typhosus* was isolated from the clot of one and in both instances blood specimens submitted within the first three weeks after onset gave definite agglutination. One of these cases proved fatal.

CHART 1. AVERAGE AGGLUTINATION TITERS WITH *B. TYPHOSUS*.

The reactions obtained in infections with microorganisms of the paratyphoid-enteritidis group, although only sixteen cases were studied, correspond to the findings reported. The average titer with the alcohol-treated suspension is more than three times that with the formalin-treated antigen. Five sera from two cases of paratyphoid fever are not included in this group because the patients had had typhoid vaccine. The average titer of these sera with the formalin-treated antigen was only slightly lower than that with the alcohol-treated suspension.

The remainder of the specimens were from patients who were not reported to be suffering from enteric disease at the time of the examination. The fact that only partial reactions were obtained in four sera from three of ten typhoid carriers, substantiates previous observations²¹ that the blood of these individuals

does not always give the characteristic agglutination reaction with typhoid bacilli. The floccular agglutinative properties were stronger with the specimens in this group, whereas in Group 4, which includes sera from 78 individuals who had previously had enteric disease but had not become carriers, the average agglutination titer was low and approximately the same with the three antigens tested. Eighty-five sera from 68 individuals who had had vaccine were tested with both antigens, and 24, or 28 per cent, gave reactions in higher dilutions with that treated with formalin. On the other hand, 16, or 18 per cent, agglutinated the alcohol-treated suspension in higher dilutions. In the remaining 45 sera, agglutination either failed to occur or was obtained in the same dilution with both antigens. Very interesting results were obtained with the serum from a woman who stated that she had not had enteric disease, but had received typhoid vaccine one month previously. The formalin-treated antigen was agglutinated in a 1:1,280 dilution, while a partial reaction with the antigen treated with alcohol occurred in a 1:20 dilution only.

The sixth group represents specimens from persons having infections other than enteric diseases, mostly undulant fever, and only those are included which have given some degree of agglutination with one or more of the antigens. Only seven of 197 sera tested with the alcohol-treated suspension and three of 146 tested with the formalin-treated suspension gave definite agglutination, while in the microscopic test, significant reactions were obtained in 42 of 173 sera tested by this method. Over 500 sera from cases diagnosed as undulant fever, which were examined during this period, failed to agglutinate *B. typhosus*. In a previous study,²² specimens from a considerable number of patients with a febrile disease other than typhoid fever were found to agglutinate the living culture of *B. typhosus* in the microscopic test.

Of the 4,000 sera examined, 106, with which reactions of diagnostic significance were obtained with one or more of the antigens, could not be included in any of the groups, or were accompanied by insufficient information to permit classification. Only 15 of these 106 specimens, however, agglutinated the formalin-treated suspension and only 24 the alcohol-treated suspension, whereas 92 of them reacted with the living culture.

SUMMARY AND CONCLUSIONS

Our study of a series of 4,000 sera has shown that the employment of macroscopic tests with two killed suspensions of typhoid bacilli, one to demonstrate the floccular or species-specific, and the other, the granular or group-agglutinative properties, usually furnishes information of greater diagnostic significance than does the microscopic test with living culture. These findings are in general accord with the results reported by other workers. Apparently, agglutination in a 1:80 or higher dilution with an alcohol-treated suspension usually indicates that the patient has typhoid fever or an infection incited by a species allied to *B. typhosus*, while a similar reaction with a formalin-treated suspension suggests one of three alternatives, that the patient has typhoid fever, has had the disease in the past, or has received typhoid vaccine. Both the granular and floccular types of agglutination have seldom been observed in high dilutions of sera other than those from typhoid fever patients.

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A STUDY OF QUINIDIN EFFECTS ON AMBULATORY PATIENTS WITH AURICULAR FIBRILLATION*

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THE value of quinidin therapy for ambulatory patients who have auricular fibrillation deserves wider recognition. Statistical surveys on the value of quinidin are devoted almost exclusively to usage of large doses on hospitalized patients. Weisman,[†] however, has pointed out recently the safety and effectiveness of the drug for the ambulatory patient. This study, completed before Weisman's work was published, was made to see if small doses had a substantial effect in converting auricular fibrillation to a sinus mechanism. Twenty-eight ambulatory patients who attended the cardiac out-patient clinic of Northwestern University were the subjects. They received quinidin sulphate in divided doses which totaled no more than 12 gr. (0.8 gm.) daily. Digitalis was also used in most cases simultaneously. Twelve or 44 per cent were restored to sinus mechanism. This result compares favorably with the reports of others who have employed large doses. To our knowledge no patient was harmed by the drug.

TABLE I
CASES RESTORED TO SINUS RHYTHM

NAME	AGE	ETIOLOGIC DIAGNOSIS	PROBABLE DURATION OF FIBRILLATION	PERIOD OF QUINIDIN THERAPY BEFORE SINUS RHYTHM	REMARKS
H. A.	29	Rheumatic	12 mo.	1 mo.	
C. V.	60	Arteriosclerosis	36 mo.	4 mo.	
T. C.	61	Arteriosclerosis	24 mo.	7 days	
S. B.	37	Syphilis	48 mo.	14 days	
J. R.	39	Rheumatic	1 mo.	7 days	
M. M.	57	Arteriosclerosis	30 mo.	7 mo.	
C. M.	47	Rheumatic?	12 mo.	3 mo.	
		Arteriosclerosis			
G. G.	63	Arteriosclerosis	24 mo.	3 mo.	
L. V.	61	Arteriosclerosis	12 mo.	14 days	
C. T.	52	Arteriosclerosis?	8 mo.	2 mo.	
		Thyroid?			
C. L.	29	Rheumatic	48 mo.	1 day	
N. R.	60	Arteriosclerosis	36 mo.	2 mo.	Died later of congestive failure
Average	50	Rheumatic 25% Arterio. 50% Syphilis 8% Doubtful 17%	24 mo.	2 mo.	

*From Cardiac Clinic of Northwestern University.

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[†]Weisman, S. A.: Auricular Fibrillation (Ambulatory Treatment with Quinidin). Arch. Int. Med. 49: 723, 1932.

An analysis of Tables I and II reveals similar age and similar duration of fibrillation in the restored and nonrestored groups. A larger proportion of restorations occurred in patients with arteriosclerosis as the etiologic factor. Table I shows that 7 of the 12 restored cases had taken quinidin for two months or longer before restoration was effected. From the well-known fact that quinidin is quickly and totally excreted, it is difficult to explain this apparent cumulative effect. Nor can the short period of the slowing of the auricular oscillation rate,* which occurred in this series, throw any light on

TABLE II
CASES NOT RESTORED TO SINUS RHYTHM

NAME	AGE	ETIOLOGIC DIAGNOSIS	PROBABLE DURATION OF FIBRILLATION	PERIOD OF QUINIDIN THERAPY TO 1-12-32	REMARKS
R. A.	48	Rheumatic	33 mo.	14 mo.	
F. G.	44	Rheumatic	13 mo.	1 mo.	
H. B.	51	Arteriosclerosis	50 mo.	14 mo.	
F. S.	62	Rheumatic and syphilis			
S. K.	42	Rheumatic	16 mo.	14 mo.	
M. B.	66	Arteriosclerosis	48 mo.	3 mo.	
R. P.	55	Arteriosclerosis	3 mo.	1 wk.	
J. M.	53	Arteriosclerosis	9 mo.	5 mo.	
G. W.	33	Rheumatic	47 mo.	11 mo.	
A. W.	60	Rheumatic	18 mo.	?	Poor cooperation
		Arteriosclerosis	24 mo.	2 mo.	Refused to con- tinue
I. K.	72	Arteriosclerosis	8 mo.	2 wk.	Refused to con- tinue
J. M.	20	Rheumatic	15 mo.	12 mo.	Death
N. R.	64	Arteriosclerosis	?	2 mo.	Failed to return
M. B.	49	Rheumatic	24 mo.	6 mo.	
F. B.	43	Unknown			
		rheumatic?	?	5 mo.	Failed to return
H. S.	28	Rheumatic	8 mo.	?	Failed to return
Average	49	Rheumatic 50% Arterio. 37% Doubtful 13%	22 mo.	7 mo.	

the mechanism. It therefore seems important to emphasize the value of continuing the administration of small nontoxic doses of quinidin for long periods of time.

SUMMARY

Small doses of quinidin sulphate are capable of establishing a sinus mechanism in a substantial percentage of patients with auricular fibrillation. There is relatively little danger in employing small doses for ambulatory patients. In some patients the drug was not effective until it had been taken for many weeks. Digitalis was used simultaneously and apparently did not interfere with the quinidin action. Small doses of quinidin were capable of slowing the auricular oscillation rate.

*The oscillation rate of auricle was obtained from needle electrodes on the anterior chest wall on 10 patients before and at 15-minute intervals after taking 3 grains quinidin. Seven of 10 showed definite slowing of auricular rate.

THE TREATMENT OF IRRITABLE COLON WITH SODIUM RICINOLEATE*†

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LARSON¹ showed that bacterial toxins could be neutralized by sodium ricinoleate in vitro. Later Dorst demonstrated that certain of the intestinal flora to which patients were highly sensitized could be detoxified in vitro and Morris and Dorst^{2, 3, 4} conceived the idea that these toxins might also be neutralized in vivo. They treated cases of irritable colon (mucous colitis) with daily doses of castor oil and later with sodium ricinoleate in gelatin capsules (soricin), obtaining very encouraging results.

They found that their patients were nearly all sensitive to autogenous vaccines made from the so-called "normal flora" of the intestinal tract, according to the method of Wherry.⁵ They also found that these vaccines could be detoxified with sodium ricinoleate, so that at least three times as much could be given without undue reaction. Detoxification did not weaken the antigenic powers. They demonstrated that, by giving these patients sodium ricinoleate orally, the skin sensitivity to heat-killed bacterial vaccines was diminished.

The purpose of this paper is to determine the effects of soricin on the symptoms and skin sensitivity to autogenous stool vaccines in cases of irritable colon. The patients were selected in the Out-Patient Dispensary and all attempts made to rule out coexisting disease, particularly intraabdominal. A diagnosis of irritable colon (or mucous colitis) was based on the accepted criteria of a history of abdominal pain, distention, constipation, a passage of excess flatus, borborygmus, nervousness, often the presence of mucus in the stools, the presence of a palpable and tender colon, and x-ray evidence of spasm.

Cultures of the stools of each patient were made. The individual strains of organisms were isolated and suspended in salt solution. They were then killed by heating to 56° for one hour. One minim of each suspension was injected intradermally and the local reaction observed twenty-four hours later. Treatment with soricin was then instituted for various periods of time.

The doses varied from 5 to 30 gr. 4 times daily. Soricin was well tolerated by a great majority of the patients. The few complaints made dealt with the large size of the capsules, occasional "sour belching," and a slight burning sensation in the epigastrium. The capsules were administered before meals and at bedtime.

*From the Department of Internal Medicine, University of Cincinnati.

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After a period of treatment, generally of two to three months' duration, the skin tests were repeated with the original suspension of organisms. Age did not diminish the intensity of the reaction to the vaccine, as was determined by comparing the effects of a newly made autogenous vaccine with a preparation a few months old, containing the same strains of organisms.

CASE 1.—A white man, aged twenty-seven, single. The patient was first seen on April 4, 1932, complaining of gas on the stomach and pain in the abdomen. During the four weeks before admission he had noticed a dull aching pain localized below the xiphoid process, coming on three or four hours after eating and lasting until he would take soda or food. Belching gave temporary relief. He had a similar attack one year previously, lasting four weeks. He was frequently constipated.

There was localized tenderness in the epigastrium just below the xiphoid process. The abdominal findings were not otherwise remarkable. A gastric analysis following ingestion of an Ewald meal revealed free HCl 77, total acids 102. The gallbladder visualized normally. Gastrointestinal x-ray studies showed spasm of the transverse and descending colon with mucus present in the latter. He was given soricin, grains 10, four times a day. Three weeks later this was increased to 20 gr. and continued until June 9, 1932.

The appetite increased, the amount of belching and flatus were definitely diminished. The pain was very much lessened and disappeared entirely in six weeks. The constipation was relieved by liquid petrolatum. He still complained of nervousness. There was no change in weight.

The organisms recovered from the stools and results obtained were as follows:

VACCINES	MAY 23, 1932	JUNE 28, 1932
1. Hemolytic bacillus coli	3.5 x 6 cm.	negative
2. Nonhemolytic bacillus coli	negative	negative
3. <i>Streptococcus viridans</i>	1.5 x 2 cm.	negative
4. <i>Streptococcus hemolyticus</i>	negative	negative
5. <i>Staphylococcus albus</i>	negative	negative

CASE 2.—A white man, aged forty-four, single, vocation electrician. The patient was first seen in October, 1929, complaining of abdominal discomfort, fullness and belching about one to two hours after meals and constipation for a period of ten years. He also complained of nervousness. His appetite was fair. There was some palpitation of the heart occasionally after meals.

Physical examination revealed tenderness on deep pressure to the right of the midline in the epigastrium and to the right of the umbilicus. The liver was felt 2 fingerbreadths below the right costal margin. Gastrointestinal x-ray series indicated the presence of mucus in the colon. Gastric analysis after ingestion of an Ewald meal revealed free HCl 35, total 59. The patient was placed on a smooth diet with bromide, belladonna, and cascara and obtained great relief from the above symptoms until April 4, 1930. He was not seen again until Dec. 2, 1930. He felt well until two months before that time, when his former symptoms returned and the presence of mucus was noticed in the stools. Physical examination showed no changes except an occasional premature ventricular contraction. The same treatment was started again, but he failed to improve. On Jan. 8, 1932, he was placed on soricin, 5 gr. 4 times a day and bromides, belladonna, and soda after each meal. From that time until May 18, 1932, his medications were changed so that for two or three weeks he would receive only soricin, 10 gr. 4 times a day, then only bromide, belladonna, and soda for a similar period, then both together. He received almost complete relief from epigastric pain and belching, and the amount of flatus was reduced while taking soricin alone. Constipation was not relieved except when taking some laxative and mucus was present in the stools from time to time. This patient had frequent head colds during the treatment and found his symptoms aggravated each time.

Organisms isolated from the stools were made into vaccines and the patient was skin-tested with each vaccine. The organisms recovered and the results obtained were as follows:

VACCINES	APRIL 17, 1932	JUNE 27, 1932
1. <i>Bacillus coli</i>	3.5 x 5 cm.	1.5 x 1.5 cm.
2. Hemolytic streptococcus	negative	negative
3. <i>Streptococcus viridans</i>	negative	negative

CASE 3.—A white man, aged forty, married, vocation, motorman. The patient was first seen on Dec. 8, 1931, with the following complaints, which he said had been present for five years: Constipation, passage of much flatus, some bloating and belching. The stools were hard and lumpy, but contained no mucus. He complained of much nervousness and frequent headaches. He came to the clinic because he thought he had cancer.

Physical examination revealed dental caries. There was slight thyroid enlargement with normal blood pressure and pulse rate. The abdomen was negative. Gastrointestinal series showed only spasticity of the descending colon with the presence of mucus. Gastric analysis after ingestion of an Ewald meal revealed free HCl 22, total 38. The patient was placed on tincture of hyoscyamus and sodium bromide and tincture of belladonna, liquid petrolatum and a smooth diet. He felt quite well for two weeks on the above regime. He was then given soricin, 5 gr. 4 times a day with no other medication. The dose of soricin was gradually increased to 10 gr. 15 gr., and 20 gr. 4 times a day. This treatment was continued until June 15, 1932. The effect of soricin upon his symptoms may be expressed as follows: There was no change in the amount of belching, he did not complain of pain at any time, and there was no change in the amount of flatus. He still complained of constipation unless liquid petrolatum was taken regularly. No mucus was seen at any time. Nervousness was somewhat lessened, and he found that he could eat some foods without discomfort which previously he could not tolerate. Organisms isolated from the stool were made into vaccines and the patient was skin tested with each vaccine.

VACCINES	MAR. 18, 1932	MAY 23, 1932
1. Gram-negative bacillus (not an acid producer)	11.5 x 17 cm.	8 x 11 cm.
2. Colon bacillus	11.5 x 17 cm.	5.5 x 6.5 cm.
3. Staphylococcus	negative	2.5 x 3.5 cm.

Twenty-four hours after the injection of the vaccines on Mar. 18, 1932, the reacting areas in Nos. 1 and 2 were red, swollen, with increased local heat. There were 2 red streaks running up the inner aspect of the arm toward the axilla, and there was tenderness in the axilla. The patient had a temperature of 100.5° F. and complained of a headache and back-ache and general malaise. It was one week before the reactions disappeared from his arm. At the second test the areas of redness were very definite, but there was no generalized reaction.

CASE 4.—A white man, aged thirty-five, vocation, policeman. The patient was admitted to the Cincinnati General Hospital on Jan. 5, 1932, complaining of pain and a heavy feeling in the lower abdomen. During the past four years he had noticed a dull aching pain just below the umbilicus, occasionally radiating to the back. It was not related to meals and was not relieved by eating or drinking. At times it would become severe but never agonizing. His appetite was good, there was frequent belching, no nausea or vomiting. His bowels moved once a day, but in addition he complained of a desire to pass stools at other times without effect. He noticed mucus in his stools frequently. He complained of a tired aching feeling most of the time and of frequent "nervous spells." Three years ago his appendix was removed but very little relief from the above symptoms was obtained. The history was otherwise negative.

Physical examination: The patient was a well-developed but somewhat undernourished white man, appearing chronically ill. The abdominal examination revealed slight tenderness

just below the umbilicus. No masses or solid organs were felt. The colon was not palpable. The red blood count was 3,100,000, hemoglobin 65 per cent (Sahli). The urine was essentially normal. The gastric analysis following the ingestion of an Ewald meal revealed free HCl 6, total 21. An oatmeal gruel fractional test meal revealed normal acidity. Stool examination was essentially negative. Basal metabolic rate was -8 and -4 per cent. Gastrointestinal series showed areas of spasm in the transverse and descending colon. A barium enema showed spasticity of the terminal third of the colon and incompetence of the ileocecal valve. The patient was placed on soricin, 10 gr. 4 times a day, starting Jan. 15, 1932. One week later this was increased to 20 gr. 4 times a day and two weeks later it was increased to 30 gr. 4 times a day and that dosage continued, except as will be described later, until June 15, 1932. He obtained very little relief from his symptoms during the first three weeks, but he then was placed on tincture of hyoseyamus, sodium bromide and tincture of belladonna in addition to the soricin for a period of two months. Soricin was discontinued during the following three weeks but was started at the end of that time at the request of the patient and the two were used in combination until June 1, 1932. He found that more relief was obtained with a combination of the two than with either one alone. The results attributable to soricin were as follows: His appetite improved. Nausea occurred on several occasions whereas very little had been noticed before treatment was started. The pain was definitely lessened. His bowels moved more regularly while taking soricin. The stools were better formed but possessed a very offensive odor. There was no change in the amount of flatus. Nervousness was definitely lessened, and he found that he could tolerate foods that previously caused him discomfort. There was no change in the weight. Stool cultures were made at the beginning and the organisms recovered were made into vaccines for skin testing. The organisms recovered and the results obtained were as follows:

VACCINES	MAR. 18, 1932	JUNE 6, 1932
1. Colon bacillus	7.5 x 16 cm.	6.5 x 7.5 cm.
2. Gram-negative bacillus (without formation of acids)	7.5 x 16 cm.	5. x 5.5 cm.
3. Streptococcus	negative	negative

Twenty-four hours after the injection of the vaccines on Mar. 18, 1932, the reacting areas of Nos. 1 and 2 were red and swollen with increased local heat. Red streaks were seen running up the inner aspect of the arm toward the axilla. There was a small tender gland palpable in the axilla. He had a slight fever, complained of headache and general malaise. At the second test the areas of redness were very definite but there was no generalized reaction.

CASE 5.—A white man, aged fifty-three, married, vocation, fireman. The patient was first seen on Jan. 3, 1930, complaining of abdominal pain and diarrhea. For two years he had suffered from a gnawing and dragging type of pain over the abdomen. This was not localized and not related to meals. He often complained of headaches over the frontal region, of extreme nervousness and 2 or 3 loose stools a day. He had not noticed blood or mucus in the stools at any time. An appendectomy had been performed ten years before admission. There had been no weight loss. The examination of the abdomen revealed the colon to be palpable and acutely tender when pressure was applied over the lower third. The cecum and ascending colon were palpable. He was not seen again until two months later, at which time he complained of the same symptoms, and in addition he had noticed mucus and blood in the stools on 2 occasions. He had lost 15 pounds in weight. Abdominal examination revealed tenderness in both lower quadrants and in the epigastrium. Gastric analysis following the ingestion of an Ewald meal showed free HCl 38, total 52. Gastrointestinal series was negative, as far as the upper gastroenteric tract was concerned. A barium enema revealed spasticity of the descending colon. Proctoscopic examination showed only 1 small hemorrhoid. He was placed on a smooth (low residue) diet with bromides and belladonna after meals. He continued on this regime with some variations until Sept. 17, 1931. During that time he showed very definite improvement for a few weeks, until the excitement of a big fire or a near accident would set him back. On September 17, he stated

that his appetite was fair, there was some belching and rumbling through the intestines, some flatus and 3 or 4 loose bowel movements every day. He was quite nervous and always tired. Soricin, 5 gr. 4 times a day was given and all other medication and diet discontinued. The soricin was increased to 10 gr. 4 times a day on Oct. 16, 1931, to 15 gr. on Jan. 6, 1932, and to 30 gr. on Jan. 20, 1932, and continued at the latter dosage until April 27, 1932. The only other medication used during that time was occasionally a half grain of luminal at night. Throughout the course of soricin treatment he experienced the same tendency to improve for a time and then a setback would follow some excessive excitement or acute upper respiratory infection. The amount of belching and flatus were increased until the larger dose of soricin was given and then there was a definite decrease. The amount of distention and bloating was somewhat lessened. The number of bowel movements and character of the stools were unchanged. The abdominal pains were slightly diminished at first and after instituting a larger dose they disappeared. There was very little change in the degree of nervousness. No change in weight was found. Organisms isolated from the stools were made into vaccines and used to test the skin sensitivity. The organisms used and the results obtained were as follows:

VACCINES	SEPT. 17, 1931	DEC. 23, 1931
1. Colon bacillus	4+	11 x 5 cm.
2. <i>Staphylococcus citreus</i>	1+	negative
3. <i>Staphylococcus aureus</i>	1+	negative

The reacting area of the No. 1 on Sept. 17, 1931, was very marked, red, swollen and tender with red streaks up the inner aspect of the arm toward the axilla. No such reaction as this was obtained on Dec. 23, 1931.

CASE 6.—A white man, aged twenty-four, married, vocation, draftsman. He was first seen on Feb. 17, 1931, complaining of "indigestion." For the past three weeks he had noticed a sharp cutting or sticking pain in the left upper quadrant, radiating to the left flank. This would appear during his meals and would last from one-half to two hours. Though his appetite was good there was fear of eating, much belching and flatus, some nausea but no vomiting. His bowels moved regularly; there was no mucus in the stools. Coffee, tea, tobacco, fried and fatty foods caused him discomfort. He had complained of a similar condition one year before. He stated that he had been jaundiced eight years ago.

On examination, the abdomen was found to be below the chest level. There was tenderness in the left upper quadrant. No masses or solid organs were felt. Gastric analysis after ingestion of an Ewald meal revealed free HCl 33, total acids 50. The stomach, duodenum, and gallbladder were entirely negative. Barium enema showed a moderate amount of mucus in the upper part of the descending colon. He was placed on bromides, belladonna and hyoscyamus and a smooth diet with temporary improvement.

He was next seen in the clinic on Jan. 25, 1932, with complaints similar to those on the previous admission.

He was placed on the same regime as before for a period of two months with slight improvement. The treatment was then discontinued and the patient given soricin, 30 gr. 4 times a day. At the end of one week the dosage was cut to 15 gr. 4 times a day and continued at that dose until May 25, 1932, when it was discontinued at the patient's request. He stated that soricin caused a burning sensation in the epigastrium soon after taking it and that the belching, rumbling sounds, and amount of flatus were all increased. He felt that it also caused him to be more constipated and said he felt much better when not taking it.

Organisms were isolated from the stool and made into vaccines. The organisms recovered and the reactions obtained from skin tests were as follows:

VACCINES	APRIL 10, 1932	JUNE 28, 1932
1. Hemolytic colon bacillus	5 x 7 cm.	3 x 4 cm.
2. Nonhemolytic colon bacillus	2.5 x 3 cm.	0.8 x 1.0 cm.
3. Small gram-positive diplococcus	2.5 x 4 cm.	1 x 1.5 cm.
4. <i>Streptococcus</i>	3 x 3.5 cm.	1 x 1.2 cm.

It is interesting to note that in spite of decrease in bacterial sensitivity, no improvement of symptoms occurred. Since that time we have had other capsules prepared with a coating which does not dissolve in the stomach. Better results have been obtained in similar cases with the new capsules.

CASE 7.—A white woman, aged twenty-five, single. This patient was first seen on April 12, 1931, complaining of pain and a feeling of tightness over the abdomen, and constipation. Her appetite was good. There was much belching, some nausea, distention of the abdomen, with rumbling sounds and much flatus. The stools were hard and in small lumps containing mucus. Occasionally she would complain of diarrhea. There were frequent headaches and backaches. She had the above symptoms for over three years before admission to the clinic. One year before the appendix, right ovary, and right tube were removed.

Examination of the abdomen revealed a right rectus scar and tenderness in both lower quadrants. Pelvic examination was negative. Gastric analysis after the ingestion of an Ewald meal revealed no free HCl, total acids 6. Free HCl was present after the injection of $\frac{1}{2}$ mg. of histamine. Gastrointestinal series revealed spasticity of the colon with excess mucus in the sigmoid region.

She was placed on antispasmodics, sedatives, mild laxatives, and a smooth diet. This regime was continued for four months without any noticeable improvement. Soricin, 5 gr. 4 times daily, later increased to 10 gr. 4 times a day was given for two months without improvement. On Jan. 22, 1932, she was placed on soricin, 20 gr. 4 times a day, with liquid petrolatum as a laxative. This was increased in two weeks to 30 gr. and continued until May 18, 1932, when she had an attack of acute catarrhal jaundice. Her appetite remained good throughout. There was no reduction in the amount of belching and flatus. She complained of much abdominal distention and bloating. The gas pains were not diminished in frequency or severity. Soricin had no effect on the constipation. There appeared to be a definite lessening in the amount of mucus. There was no change in weight.

A stool culture in August, 1931, revealed an overwhelming growth of colon bacillus. This was made into a vaccine. A marked reaction was obtained on intradermal injection. Unfortunately, the vaccine was lost so that no test was made after treatment with soricin.

CASE 8.—A white male, aged thirty-two years, single. This patient was admitted to the hospital on Dec. 3, 1930, complaining of nervousness and bowel trouble. These symptoms had been present for thirteen years and during that time the patient had frequently noticed "yellow mucus clots" in the stools. He took a cathartic or an enema almost daily during that time. In 1924, a colostomy was performed and the colon irrigated because of a "mucous colitis." This gave improvement of symptoms at that time but after closure of the wound the same symptoms returned. He was quite excitable and worried a great deal about his condition.

On physical examination the abdomen was negative. Gastric analysis after the ingestion of an Ewald meal gave free HCl 38, total acids 53. Stool examination was negative for mucus, blood, and parasites. The patient was not seen again for a year and returned complaining of the same symptoms. A gastrointestinal x-ray series showed some redundancy of the hepatic flexure and spasticity of the descending colon. Gastric analysis revealed free HCl 48, total acids 70. He was placed on soricin, 5 gr. 4 times a day, until June 1, 1932. His appetite was good throughout. There was no belching, nausea, vomiting, or abdominal pain at any time. Constipation was relieved to some extent by liquid petrolatum, the soricin appearing to make him more constipated. The nervousness was somewhat lessened. There was a loss of 6 pounds in weight. Organisms cultured from the stool were made into vaccines and used to determine the skin sensitivity. The organisms recovered and results obtained were as follows:

VACCINES	MAR. 17, 1932	JUNE 28, 1932
1. Hemolytic colon bacillus	negative	negative
2. Nonhemolytic colon bacillus	5.5 x 6.5 cm.	3 x 4 cm.
3. Streptococcus	negative	negative
4. Staphylococcus	negative	negative

CASE 9.—A white man, aged forty-one years, married. The patient was first seen on Feb. 1, 1932, complaining of constipation. For the past nine years he had been troubled with constipation, passage of mucus in the stools, nausea, belching, and a dull pain under the left costal margin. There was also considerable nervousness and sweating. During the last eight weeks before admission to the clinic all of his symptoms were much intensified.

On physical examination the abdomen was tender in both lower quadrants. The descending colon was palpable and tender. Gastric analysis following the ingestion of an Ewald meal revealed no free HCl. Following an injection of $\frac{1}{2}$ mg. of histamine the free HCl was 62.0. Blood Wassermann was negative. A gastrointestinal x-ray series showed narrowing of the descending colon with absence of the haustra. A barium enema revealed spasticity of the distal third of the colon. The gallbladder visualized normally. A proctoscopic examination revealed only external and internal hemorrhoids. On Feb. 12, 1932, he was placed on bromides, hyoseyamus, belladonna, and soda with liquid petrolatum as a laxative. This regime was continued until April 8, 1932, without noticeable improvement. Soricin, 10 gr. 4 times a day was then added to the above medication, and he continued with that regime until May 27, 1932.

The appetite was unchanged. Belching was lessened but he complained of belching after taking the soricin, stating that the gas had a bad odor. There was definite reduction in the severity of the abdominal pain. There was some reduction in the amount of mucus. Constipation was relieved by liquid petrolatum. He still complained of much nervousness. There was no change in weight.

Organisms isolated from the stool were incorporated into vaccines and used to test the skin sensitivity. The organisms recovered and the results obtained were as follows:

VACCINES	JUNE 4, 1932	JUNE 28, 1932
1. Hemolytic colon bacillus	2.5 x 3.5 cm.	negative
2. Nonhemolytic colon bacillus	2.5 x 3.5 cm.	1.5 x 2 cm.
3. Streptococcus	negative	negative

CASE 10.—A white man, aged twenty-seven, single. This patient was first seen in March, 1929, complaining of pain in the abdomen. This was of a dull aching character located in the right upper and lower quadrants, appearing about an hour and a half after each meal. It was relieved slightly by soda and completely by a bowel movement. The pain was first noticed two years before admission. He was habitually constipated and had noticed mucus in the stools for several years. He had lost 17 pounds in weight.

The physical examination was essentially negative throughout. A gastrointestinal x-ray series was negative. The gallbladder visualized faintly after the oral and intravenous administration of iodeikon. On May 31, 1929, a cholecystectomy and appendectomy were performed following an acute attack of pain in the right upper quadrant. A chronic cholecystitis without stones was found at operation; the appendix was normal.

He was not seen again until Jan. 16, 1931, at which time he complained of the same symptoms as before operation. A gastric analysis after the ingestion of an Ewald meal revealed free HCl 48, total acids 70. A gastrointestinal x-ray series showed only hyperperistalsis. He complained of a dull pain in the right half of the abdomen, much belching and flatus, constipation with the passage of mucus in the stools, and nervousness. Various types of treatment were tried without results. In December, 1931, a series of elimination diets was used to determine the presence of food allergy with negative results. On Jan. 28, 1932, he was placed on soricin, 15 gr. 4 times a day and liquid petrolatum. In two weeks the soricin was increased to 30 gr. 4 times a day and maintained at that dosage until June 1, 1932, with the exception of ten days during May when he was in the hospital for another condition.

The appetite was poor throughout, there was slight lessening in the amount of belching and flatus. The abdominal pain was diminished. No mucus was seen in the stools after treatment with soricin was started. Constipation was controlled with liquid petrolatum. The nervousness was definitely lessened. During the ten days he was in the hospital and not receiving soricin, he stated that his symptoms were much increased in severity.

Stood cultures were made and used to test the skin sensitivity. The organisms used and results obtained were as follows:

VACCINES	MAR. 23, 1932	JUNE 6, 1932
1. <i>Bacillus mucosus</i>	4 x 5 cm.	negative
2. <i>Bacillus coli</i>	5.5 x 11 cm.	3.5 x 5.5 cm.
3. <i>Staphylococcus</i>	negative	negative
4. <i>Streptococcus</i>	2 x 2 cm.	negative

The reacting areas of Nos. 1 and 2 on Mar. 23, 1932, were red, hot, and swollen, and there was pain in the corresponding axilla. No such reaction was noticed with the second test.

CASE 11.—A white man, aged forty-one, married, vocation, engineer. The patient was first seen on Nov. 24, 1931. For the past five years he had complained of a dull aching pain radiating across the lower abdomen, coming on about one-half hour after each meal and lasting a few minutes, followed by much belching. The appetite was good, there was much rumbling of the intestines. Generalized abdominal soreness and constipation were present. No mucus or blood was seen in the stools. There was a history of syphilis eight years ago, for which he had received active treatment.

Physical examination: On physical examination there was abdominal tenderness, and the descending colon and sigmoid were palpable and tender. X-ray examination revealed moderate spasm of the transverse and descending colon. Gastric analysis following ingestion of an Ewald meal gave free HCl 20, total acid 36.

He was placed on a smooth diet of Alvarez, liquid petrolatum and tincture of belladonna for one month without noticeable improvement. On Dec. 30, 1931, soricin, 5 gr. 4 times a day, was started, all other medications being discontinued. The soricin was increased to 10 gr., then 20 gr., then 30 gr. 4 times a day, allowing two weeks to elapse between each increase, and the latter dosage continued until May 25, 1932. Liquid petrolatum was given as a laxative. Pain was much reduced after the first two weeks' treatment with soricin and was practically absent until May 5, 1932. Since then, it has been present at intervals, though of little consequence. The amount of belching was definitely lessened for four months and then returned. There was some diminution in the amount of flatus. Soricin had no effect on the constipation. His appetite remained the same throughout, but there was a loss of 12 pounds in weight.

Organisms isolated from the patient's stool were made into vaccines and the patient was skin tested. The organisms recovered and results obtained were as follows:

VACCINES	APR. 20, 1932	JUNE 8, 1932
1. <i>Bacillus coli</i>	3.5 x 4 cm.	4 x 4 cm.
2. <i>Staphylococcus albus</i>	4.5 x 5 cm.	4 x 4.5 cm.
3. <i>Streptococcus</i>	negative	negative

There was no apparent change in the reactions to the vaccines after treatment with soricin for a period of approximately five months.

CASE 12.—A white man, aged twenty-eight, single. The patient was first seen on Mar. 14, 1932, complaining of pains in the abdomen of two months' duration. They were cramp-like in character and at first confined to the epigastrium. Later they began to localize in the right upper quadrant. For two days they were accompanied by nausea and vomiting and aching over the entire body. At times there was some radiation to the right shoulder blade. His appetite was good. There was some fullness after eating during the past three or four months. Tarry foods and fried foods caused discomfort. He has been constipated since the onset of this illness. There has been a loss of 15 to 20 pounds in weight.

On physical examination there was increased resistance over the right half of the abdomen but no tenderness. Gastric analysis after the ingestion of an Ewald meal revealed free HCl 47, total acid 62. Gastrointestinal x-ray studies showed irritability of the duodenum and slight downward displacement of the hepatic flexure of the colon. The gall-

bladder visualized normally. The patient was placed on a bland diet and hyoseyamus and bromides with liquid petrolatum for the constipation. He continued on this regime for five weeks without noticeable improvement. At this time the above medication was discontinued except for the petrolatum, and he was given soricin, 10 gr. 4 times a day. This was increased to 15 gr. and then to 20 gr. 4 times a day. The belching was definitely lessened and he had relief from nausea. There was a marked diminution in the abdominal pain.

The organisms cultured from the stools used and results obtained were as follows:

VACCINES	MAY 28, 1932
1. Hemolytic bacillus coli	3.5 x 6.5 cm.
2. Nonhemolytic bacillus coli	negative
3. <i>Staphylococcus albus</i>	negative

The patient did not return to the clinic for a second determination of sensitivity.

SUMMARY

Twelve patients exhibiting the symptoms which are attributed usually to an irritable colon have been treated with sodium ricinoleate. Prior to initiation of this treatment, these patients had failed to respond to a bland diet and antispasmodics. It is to be noted that the cases reported by Dorst and Morris⁴ had all been under treatment for a period of six months or longer. Some of the inconclusive results obtained with certain of the cases included in this series are reported after only three months of treatment and would probably respond to persistent therapy. The general results may be outlined as follow:

There was diminution of the pain and belching in the majority of the cases. The amount of flatus was diminished in five. As has been previously shown, constipation was not affected. Mucus in the stools was appreciably lessened or disappeared in 50 per cent of those who showed this symptom. It is important to note that those patients who did not show clinical improvement in the treatment with soricin had, with one exception, definite gastric symptoms following the ingestion of the drug. This demonstrates the necessity for having more adequate enteric coating, since the sodium ricinoleate, if liberated in the stomach and acted upon by gastric secretions, gives rise to distressing symptoms.

The skin sensitivity to organisms from the intestinal flora was reduced materially in 9 out of 10 cases. In two cases it was impossible to obtain sensitivity records at the end of treatment.

CONCLUSIONS

1. It is felt that soricin is of definite benefit in the treatment of irritable colon.
2. There is a decrease in the skin sensitivity to bacterial vaccines following treatment with soricin.

This tends to support the theory of Dorst and Morris, that soricin neutralizes bacterial toxins in vivo.

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THE EFFECT OF SALYRGAN AND X-RAY ON THE RATE OF DISAPPEARANCE OF THROMBOPHLEBITIC EDEMA*

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IN A PREVIOUS publication,¹ we reported a method for producing edema in the hind legs of experimental animals comparable to that seen in man following deep thrombophlebitis. Chemical study of the edema fluid revealed a rather high total protein content, the values ranging from 1.25 to 2.34 per cent. It was indicated that this accumulation of protein-rich fluid in the tissue spaces might be a factor in the perpetuation of postphlebitic indurations.

In possession of a method to produce such an edema, it was possible to evaluate the effect of measures which might hasten the disappearance of the acute edema in order to prevent chronicity. Consequently, the disappearance rate of experimentally produced edema in untreated animals was compared with that in dogs given a mercurial diuretic or x-ray exposure. In the first series of experiments, salyrgan was injected to determine the possibilities of mobilizing the edema fluid by this method.² The effect of x-ray was studied because of the excellent results reported from more or less empirical clinical use of such treatment.³

METHODS OF EXPERIMENTATION AND RESULTS

Edema was produced in the leg of the dog by the method previously described, namely, the injection of a concentrated tissue extract (Fibrogen, Merrell) peripherally into the femoral vein. This results in extensive peripheral, intravascular thrombosis, and massive edema of the extremity. The dosage of fibrogen varied from $\frac{1}{2}$ to 1 c.c. and the degree of edema was usually roughly parallel to the quantity of fibrogen injected. Careful measurements of the circumferences of the limbs were made before and at varying intervals after the injections. Readings were taken at four levels: through the middle of the thigh, the popliteal fossa, the middle of the leg, and the foot. Subtracting the dimensions before injection from those after it, gave a measure of the increase in circumference due to edema. Because the variations in

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diameter at the thigh and knee greatly exceeded those of the leg and foot, a sum of the four differences, rather than an average, was taken as an index of the amount of edema. The measurements were almost entirely made by the same person, who often was not informed which of the animals were controls, and which experimental. Comparison of the circumferences of the two hind legs of the same animal revealed the margin of error in the measurements to be very small.

CONTROLS

In a series of twenty animals, edema was produced by the method described, and measurements taken at intervals over a period of two weeks. The results are shown in Chart 1. The heavy line represents the average curve in this series illustrating the rate of disappearance of edema in untreated animals. In all other respects these dogs were kept under the same conditions as were the ones in the following groups.

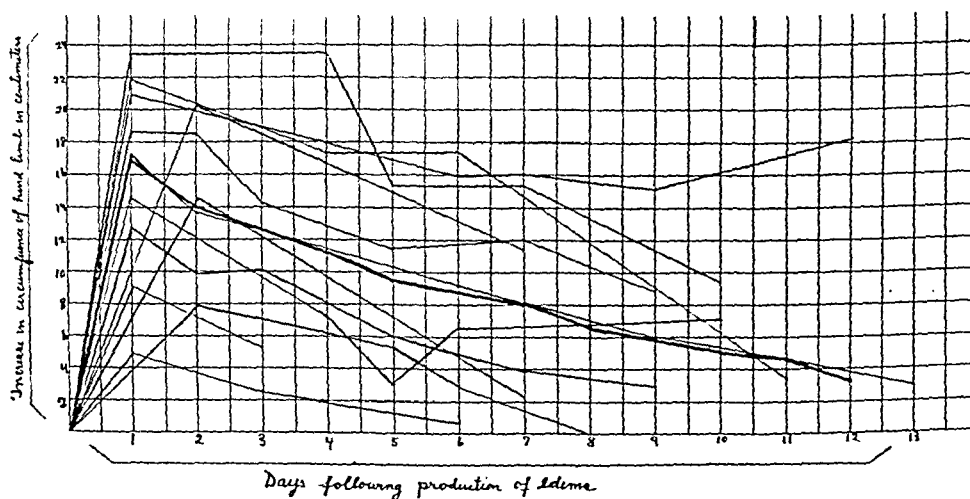


Chart 1.—The normal rate of disappearance of experimentally produced edema. The heavy line represents an average curve of increase and decrease of a hind limb in the untreated animal.

SALYRGAN EXPERIMENTS

In this series of twenty animals edema was produced in exactly the same way as above. At varying intervals after the edema reached its height, salyrgan, in doses of 1 c.c., was injected intravenously into the opposite extremity. In some experiments the injection of salyrgan was repeated. Salyrgan was used alone, without ammonium chloride or other acidifying agent. The results of these experiments are seen in Chart 2. The arrows indicate the time at which the salyrgan was given. The heavy black line represents the composite curve for this series of experiments. It will be seen from these curves that a rapid diminution in the circumferences of the edematous limbs occurred immediately following the salyrgan injection. It is also seen that repetition of the injection usually failed to produce a second sharp reduction in the degree of edema. The disappearance curve of the salyrgan treated animals is super-

imposed upon that of the controls in Chart 4. In this figure the average maximum circumference increase of each series represents 100 per cent, and the curve shows the percentage daily decrease in the respective groups. From this it will be seen that the greatest acceleration of disappearance time following salyrgan administration occurred during the first few days, after which the two curves run an almost parallel course.

It must be stated that no attempt was made to regulate the fluid intake of the experimental animals in this group, because no such restriction had been made in the control series. It is quite likely that, following the diuresis and consequent dehydration which resulted from the salyrgan injection, the animals consumed large quantities of water, and thus, in a measure, replaced the fluids lost. It seems reasonable to suppose that, had the fluid intake been restricted, as has been done in the case of human beings subjected to diuresis for the purpose of mobilizing excessive tissue fluid,² the rate of disappearance of the

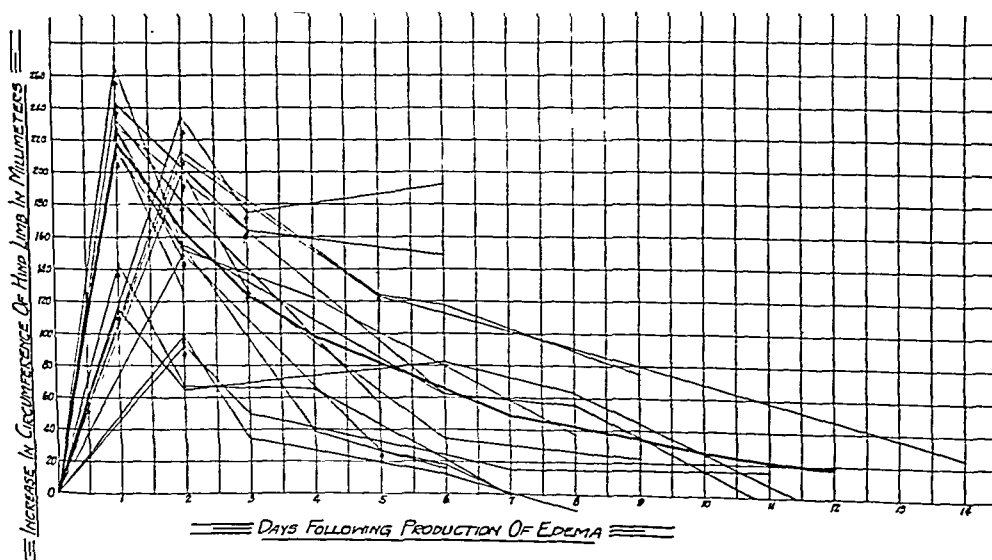


Chart 2.—The rate of disappearance in experimentally produced edema, when salyrgan was injected at the height of the edema. The arrows indicate the injections of salyrgan. The heavy line represents the composite curve for this series. It will be seen, that the injection of salyrgan is followed by a rapid diminution in the circumferences of the edematous limbs. The second injection does not seem to be as efficient as the first.

edema would have been more rapid. These experiments at least serve to indicate that diuresis produced by the administration of mercurial diuretics is capable of mobilizing edema fluid from the extremities of animals in which experimental postphlebitic edema had been produced.

X-RAY EXPERIMENTS

The therapeutic use of x-ray has recently been advocated for the treatment of early edemas of postphlebitic origin, notably by Halban, of Vienna.³ Excellent results are claimed for this treatment. A series of experiments was performed to determine the relative effect of x-ray upon the rate of disappearance of postphlebitic edema in the laboratory animal. Edema was produced as in the other series, and at the height of the edema, the limbs were exposed

to x-ray in doses varying from 100 to 400 r. Measurements were made of the circumferences before and after irradiation, and the results plotted on Chart 3. From these curves it may be seen that a definite diminution in the edema oc-

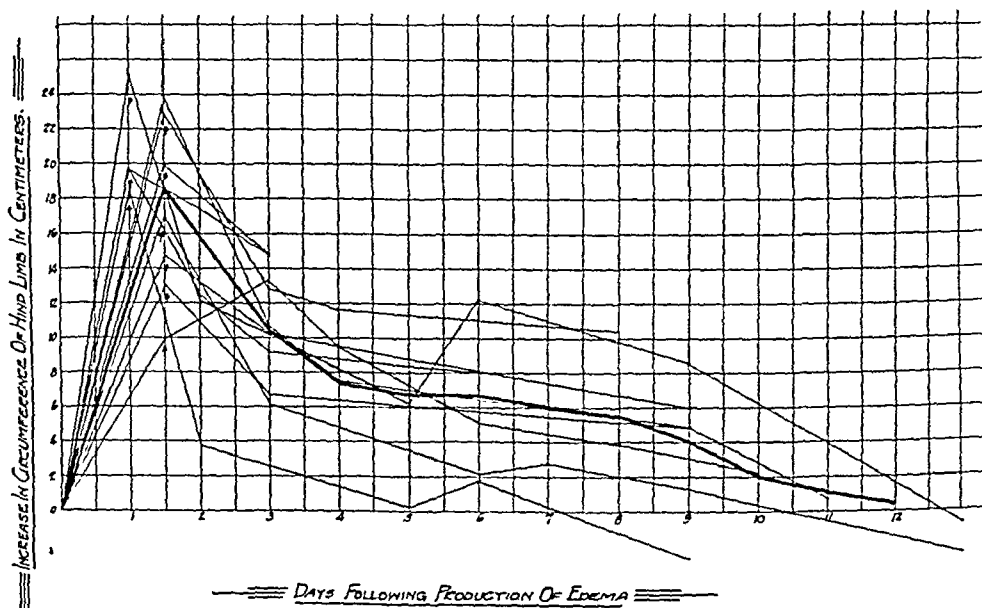


Chart 3.—The rate of disappearance of experimentally produced edema following exposure to roentgen ray. The heavy line represents the average curve. The exposure was not always given at the height of edema.

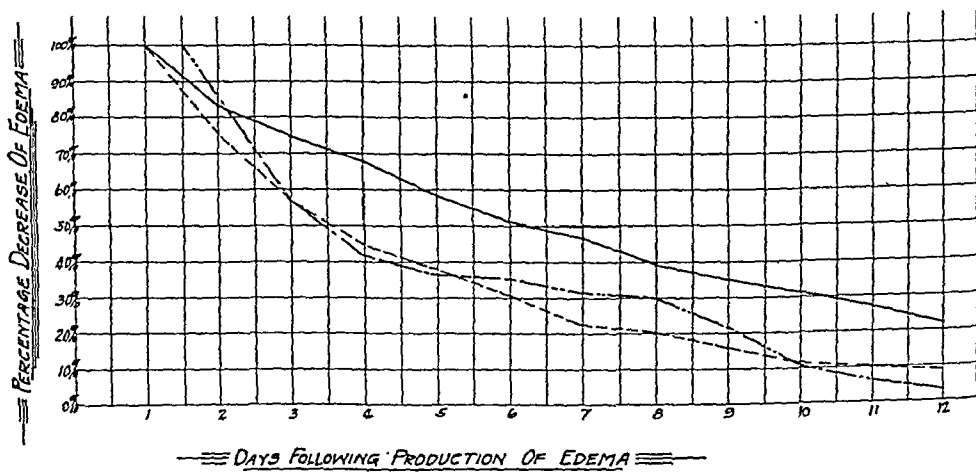


Chart 4.—Composite graph of the three series of experiments. The straight line represents the normal rate of disappearance of edema. The interrupted line shows the disappearance of edema in animals treated with salyrgan. The interrupted line with short and long dots illustrates the diminution of edema following exposure to roentgen ray. The diminution of edema is calculated as a percentage decrease from the height of edema, the maximum increase of each series being 100 per cent. The curve for the x-ray animals coincides very closely with the curve of the animals treated with salyrgan. Both of those curves dip more rapidly than the control curve.

curring immediately following the x-ray exposure. This was occasionally followed by a slight secondary increase. In these animals, as in the others mentioned, no attempt was made to limit the fluid and salt intake. In Chart 4 are

seen the superimposed disappearance curves of all three groups, shown in percentual decrease, rather than in units of measure. The curve for the x-ray animals coincides very closely with that for the salyrgan treated ones. Both of these revealed more rapid disappearance than in the controls.

DISCUSSION

An early disappearance or diminution of the acute thrombophlebitic edema is obviously desirable, if permanent tissue changes are to be prevented or minimized. The response of such an edema to salyrgan, a mercurial diuretic, offers additional evidence of the extrarenal action of this drug. Its clinical use, combined with maximal elevation of the limb and restriction of fluid and salt intake, and followed by gradual exercise and massage, has given gratifying results.² The attempt to diminish pain and edema of the limb with x-ray has a purely empirical basis, and our preliminary results would indicate that further work on optimal dosage, filtration and time interval between treatments, is justifiable. The mechanism of its action is obscure, and one can only speculate as to whether it exerts an influence upon the lymphatic flow, or causes a breaking down of lymphocytes and leucocytes with the liberation of proteolytic enzymes and digestion of the protein-rich exudate to a more absorbable form, or results in an ionization of the tissue fluids, or systemically affects fluid mobilization. A more exact knowledge of the effects of roentgen radiation upon normal tissues must be available⁴ before an analysis of effects in pathologic conditions can be undertaken. Possibly several of the above mentioned factors are involved in the more rapid disappearance of an acute lymphangitis or periphlebitis such as we have been able to observe clinically.⁵ One of us has observed beneficial effects in the treatment of latent infections in varicose veins by means of x-ray.⁶ Further clinical and experimental studies are under way to determine the value, limitations and possible dangers of x-ray treatment in acute thrombophlebitis.

SUMMARY

Acute thrombophlebitic edema has been produced in dogs by the injection of a concentrated tissue extract into the femoral vein, and the normal rate of disappearance of the fluid has been determined in a control series of twenty animals. In a second series of twenty dogs, edema was produced in the same manner. After the edema had reached its height, salyrgan, in doses of 1 c.c., was injected intravenously. A rapid diminution of the edema followed, which definitely exceeded the normal rate of disappearance. In a third series of twelve dogs, the edematous limbs were treated with x-ray. The curve of disappearance of the edema in this group was similar to that in the salyrgan treated animals. A possible interpretation and the clinical application of these findings has been briefly discussed.

We wish to express our sincere gratitude to Dr. Earl R. Crowder of the Department of Radiology, Northwestern University Medical School, who gave the roentgen treatments and offered many valuable suggestions during the course of experiments.

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A COMPARATIVE STUDY OF CERTAIN XANTHINE DIURETICS*

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INTRODUCTION

CAFFEINE, theophylline, and theobromine have been made the subjects of extensive studies by many investigators, but no reliable quantitative clinical experiments concerning the comparative diuretic effects of the various xanthine derivatives in graduated doses have been reported. With the exception of one quantitative report in German,¹ there are no published reports of experimental work on laboratory animals with the newer theobromine and theophylline derivatives administered in graduated doses. The conflicting statements in the literature, and numerous inquiries concerning the diuretic powers of the newer derivatives of the methylxanthines prompted these investigations of theophylline calcium salicylate (phyllicin), theobromine calcium salicylate (theocalcin), theophylline sodium acetate (theocin soluble), theobromine sodium salicylate (diuretin), theophylline, and theobromine.

When the studies were begun, the primary objects were to determine what influences these agents, in graduated doses, have on diuresis, and to establish relatively safe maximum doses, so far as determinable effects on the gastrointestinal tract and on the kidneys are concerned. During the course of the work several additional factors presented themselves, the chief one being the influence exerted by these agents on the excretion of uric acid. However, during these studies no attempts were made to discover how these drugs actually increase the uric acid content of the urine, or by what mechanism they influence diuresis,² controversial points in the literature.

The consensus of opinion appears to be that, although xanthine possesses little diuretic power, certain derivatives containing the xanthine nucleus are among the most powerful diuretic drugs. Underhill and Paek³ state that caffeine, theobromine, and theophylline are without doubt the peers of drug

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diuretics for man, and that rabbits likewise are quite responsive to the diuretic influences of these compounds. These investigators found that intravenous injections of the foregoing xanthine derivatives do not increase the volume of urine output. Of the dimethylxanthines, theophylline and theobromine are the more active, while caffeine is the most effective of the trimethylxanthines. Some investigators consider theobromine to be more reliable and less prone to produce disagreeable nervous reactions, while others claim it is apt to prove irritating to the kidneys. Theophylline is credited by many with being a more prompt diuretic, and its diuretic action is supposedly of shorter duration. However, this drug is said to be more irritating to the kidneys and the gastrointestinal tract.

Caffeine, theophylline, and theobromine may fail to promote diuresis if there is not an excess of water in the tissues, as with rabbits which have been kept on a dry diet for a long period of time. These drugs, apparently, hasten the excretion of excess water.⁴ On the other hand it has been found that caffeine may fail to increase diuresis even when water is at the disposal of the animal. Schroeder,⁵ in 1886, demonstrated that caffeine invariably acts as a diuretic in rabbits if the nerves supplying the kidneys are first sectioned, or if paraldehyde or chloral hydrate are administered first to the animals, measures thought to prevent an interfering vasomotor stimulation. In the dog, diuresis is always obtained if the kidneys are denervated first or the vasomotors paralyzed, and section of the vagus nerves results in increased diuresis or induces it when absent. These observations have led to the conclusion that the vagus nerves carry inhibitory fibers to the secretory cells of the dog's kidney, and that caffeine stimulates these fibers.⁶

Wallace and Pellini⁷ showed that theophylline, theobromine, caffeine, and "diuretin" not only failed to produce a diuretic action on dogs which had been kept on a fixed food and water intake, but actually demonstrated a decided decrease in the daily output of urine which often amounted to 50 per cent of the normal volume. They found also that the sodium chloride content was diminished. These workers suggested that there is a shift of water and sodium chloride from the circulating blood to the tissues, and that caffeine and its allies bring this about through the production of a dilatation and an increased permeability of the capillaries in the splanchnic organs and muscles, these effects overshadowing the kidney action in animals which are in water balance. The diuretic effects, then, appear when there is an excess of water in the body, or when the tissues give off some of their stored water. Widmer⁸ claimed that the efficiency of caffeine as a diuretic is proportional to the amount of body fluid.

Most writers state that the effects of theobromine on the kidneys are practically identical with those of caffeine. Solis-Cohen and Githens⁹ claim that theobromine differs from caffeine in not stimulating the vasomotor and the inhibitory secretory nerves of the kidneys, and that it consequently produces diuresis constantly in dogs and in rabbits. Sasaki¹⁰ demonstrated that the continued administration of theobromine increased water excretion in rabbits only when these animals were kept on a wet diet; but that chloride excretion is increased

also on a dry diet, i.e., independently of a diuretic action. Hellin and Spiro¹¹ found that caffeine is effective in pure tubular nephritis, but that it fails in glomerular and general nephritis. They reported that theophylline and theobromine are more effective. Pearce, Hill and Eisenbrey,¹² and Schlayer and Hedinger¹³ confirmed these observations. Many consider these findings as important evidence that the diuretic effect of caffeine is exerted on the glomerular circulation and filtration, and not on the tubular epithelium. Solis-Cohen and Githens¹⁴ state that when albumin is present, the total amount is generally not increased by the ingestion of caffeine, so that the percentage is diminished because of the increased output of water. These writers state further that occasionally the total amount of albumin may be increased. Sollmann¹⁵ states that caffeine may increase the albumin in nephritis. According to Vinci¹⁶ large doses of caffeine (40 mg. per kilo), long continued especially, produce definite nephritic changes; that dogs are more susceptible than rabbits; but that no lesions have been observed following doses of from 5 to 10 mg. per kilo in man.

Sollmann and McComb¹⁷ state that caffeine in ordinary doses does not appear to produce any demonstrable injury even in nephritic subjects, but that in larger doses it may increase albuminuria. They condemn the continuous use of theophylline even in moderate doses. Emerson¹⁸ reported no increase in albumin in acute or chronic nephritis following the administration of theobromine. Miller¹⁹ is of the opinion that theophylline and theobromine may cause irritation of the stomach and kidneys. Pouchét and Chevalier²⁰ claim that theophylline in large doses injures both the glomerular and the tubular epithelia. Chevalier²¹ reported the presence of toxic impurities in commercial samples of theobromine. Vieth and Leube¹ determined the most effective daily doses of theobromine, "diuretin," "theocalcin," "phylllein," etc., in rabbits. They concluded that of the acid groups introduced in position one of the theobromine molecule, only the acetyl group increased diuresis. Bürgi's²² assumption that the diuretic powers of the xanthine derivatives are enhanced by salts was confirmed by these investigators in their experimental work with certain salicylates. They demonstrated that the calcium salts of the xanthine derivatives may influence diuresis in rabbits if this metal is combined with salicylic acid.

Stewart²³ observed that, in man, theobromine calcium salicylate produced less gastric irritation than theophylline and theobromine sodium salicylates; and that this drug may be administered daily for months without untoward symptoms. Selig²⁴ is one of the very few writers who has reported the effect of theobromine calcium salicylate on the volume of urine excreted by man. He observed that this drug induces diuresis. Cushny²⁵ states that, when large doses of theobromine are taken for some time, it is prone to act on the stomach and cause loss of appetite and nausea; and that theophylline in a number of cases has had a deleterious action on the stomach, and has caused renal irritation and epileptiform convulsions. He states further that caffeine does not injure the kidney even in large doses and over prolonged periods, and that its administration, therefore, in renal disorders is without risk of increasing the lesions. Solis-Cohen and Githens²⁶ state that in normal men it has been found that the

average increase in the twenty-four-hour urine resulting from a single dose of 0.5 gm. of caffeine is 42 per cent; and that in rabbits this is true if the doses are below 10 mg.

The literature contains numerous reports of studies concerning the effects of ingested caffeine and other methylated xanthines on the excretion of uric acid, but the reports recorded vary widely. Some investigators state that caffeine increases the uric acid content of the urine; others have found no change in the excretion of uric acid. The weight of published evidence in the earlier literature favors the former view. In 1850 Lehmann²⁷ reported that no traces of caffeine were found in the urine after the administration of caffeine. The isolation of methylated purines from normal human urine in 1895 was followed by the reports of Albanese²⁸ that the ingestion of caffeine and of theobromine in dogs resulted in the appearance of monomethyl-xanthine in the urine. During the period 1885 to 1905 Bodzynski and Gottlieb,^{29, 30} and Krüger and Schmidt^{31, 32} obtained similar results with theobromine and with theophylline when administered to dogs or rabbits. Taylor³³ reported an increase in uric acid following the drinking of coffee. Burian and Schur³⁴ found that the administration of caffeine failed to produce any effect on the amount of uric acid in the urine. Hess and Schmoll³⁵ reported an increase in the uric acid after the drinking of coffee and tea. Haig³⁶ observed an increase in the amount of uric acid following the ingestion of coffee.

In 1916 Benedict³⁷ reported an increase in uric acid following the administration of caffeine, and Brugsch and Schittenhelm³⁸ and Mendel and Wardell³⁹ obtained similar results with caffeine, coffee and tea. These investigators believe that the increased uric acid excretion is possibly due to the demethylation and subsequent oxidation of at least a part of the ingested methylxanthines. The investigations of Mendel and Wardell showed that there is a marked increase in the excretion of uric acid following the ingestion of caffeine, coffee and tea, and that the increase is apparently proportional to the quantity of caffeine ingested. They found that the increase in the amount of uric acid excreted is equal to the quantity of uric acid which would be obtained by the demethylation and subsequent oxidation of from 10 to 15 per cent of the ingested caffeine.

Clark and deLorimer⁴⁰ found that caffeine increased the excretion of uric acid in the urine, but that the primary increase was usually followed by a decrease in the excretion; and that theobromine diminished the rate of excretion of uric acid although there was no apparent change in the rate of its formation. These investigators concluded that the increased uric acid excretion following the administration of caffeine is probably due to direct oxidation of the methylxanthines, and suggested that in a general stimulation of metabolism, the kidney cells react first to the increased endogenous uric acid causing an increased excretion, and subsequently the kidney excreting cells react to the foreign and more potent compounds, caffeine or various demethylated derivatives, resulting in a specific excretion of these substances to the partial exclusion of uric acid. Cushny⁴¹ states that the uric acid of the urine is not increased by caffeine, theobromine, or theophylline. Bastedo⁴² states that caffeine in

large doses increases metabolism; and that caffeine tends to lose its methyl groups as it passes through the organism, with the resulting formation of monomethylxanthines, xanthine and urea which are excreted in the urine. Means, Aub, and DuBois⁴³ demonstrated a definite increase in the basal metabolism in man following doses of from 0.5 to 0.7 gm. of caffeine. Myers and Wardell⁴⁴ observed an increase in the urinary uric acid after the ingestion of caffeine and theophylline, but not after theobromine. These workers found that when about 300 mg. of theophylline was administered there was an increase of 100 mg. in the uric acid excreted in the urine, a quantity which represents the transformation of 33 per cent of the theophylline ingested. Taylor and Rose⁴⁵ confirmed the conclusion of Burian and Schur⁴⁶ that part of the nitrogen of ingested purine is excreted at once as uric acid, while part meets an unknown fate. Koehler,⁴⁷ and Folin, Berglund and Derick⁴⁸ have also confirmed Burian and Schur. Mendel and Lyman⁴⁹ found that hypoxanthine ingested by man caused an excretion of 60 per cent of the equivalent amount of uric acid, while xanthine caused 50 per cent, guanine 25 per cent, and adenine 34 per cent. Rose⁵⁰ concluded that caffeine and its allies are excreted as such, without the formation of uric acid, and that it was uncertain whether they have any effect on the excretion of uric acid from other sources. Magath⁵¹ claims that the uric acid in the urine is definitely increased after the administration of caffeine.

According to Wiechowski,⁵² in dogs, the increased output of uric acid after its parenteral introduction has never been reported higher than 21 per cent. For rabbits, Burian and Schur⁴⁶ reported 16 per cent, Wiechowski⁵² 5.6 per cent, Bendix and Schittenhelm⁵³ 18 per cent, and Croftan⁵⁴ from 11 to 17 per cent.

Homer⁵⁵ found that when dogs are placed on diets which are rich in meat, appreciable amounts of uric acid were eliminated in the urine, 500 gm. of meat per day yields from 0.2 to 0.3 gm. of urinary uric acid in twenty-four hours.

Burian and Schur⁵⁶ observed an increase in the hourly excretion of uric acid in dogs which had been given uric acid intravenously, the increase being most marked during the first hour or two. Later there was a fall, and the twenty-four-hour uric acid output was not increased. These investigators explained this on the basis that the rate of the normal destruction of uric acid in the body of the dog was disturbed during diuresis, probably due to circulatory changes; and conversely not due to an increased production of uric acid.

Morris and Rees,⁵⁷ who used isotonic and hypertonic solutions of sodium chloride and sodium sulphate with rats and with rabbits, observed that strenuous diuresis increased the total uric acid output in twenty-four hours, and that, as with urea, the output of uric acid is affected by the volume of the urine excreted more than any other factor. They found no evidence of uric acid arising directly from increased kidney activity. Their first series of observations were made with rats and extended over a premedication period of three days and a two-day period following the administration of one dose of the drug, given on the fourth observation day. They found with the sodium sulphate rats an average increase in uric acid output of 59 per cent at the end of the day on which the drug had been administered, as compared with the

average of the three premedication days. The chloride content was increased also. The sodium chloride rats showed a 42 per cent gain in uric acid output. The lowest gain in both groups of animals was 2 per cent, and the highest 66 per cent. The average twenty-four-hour output of uric acid for the sodium sulphate animals was 0.87 mg. per 100 gm. of body weight, and for the sodium chloride animals 0.65 mg. Hourly collections through twenty-four hours made with a second group of rats disclosed the fact that isotonic or hypertonic sodium sulphate solutions cause a large excretion of uric acid in the first or second hour with a compensatory drop later; while the sodium chloride solutions tend toward a more even level in output. The average values obtained for the twenty-four-hour uric acid output were: (1) with sodium sulphate, 0.88 mg. per 100 gm. of body weight, and (2) with sodium chloride, 0.98 mg. If these animals are grouped according to the concentration of the ingested fluids, hypertonic solutions produce 1.01 mg., and isotonic solutions, 0.81 mg. Rats of a third series were given three doses of the drugs at two-hour intervals. The sulphate animals showed a drop in the level of uric acid excreted with each administration, whereas the chloride animals showed a tendency to maintain a more uniform excretion. However, the total six-hour excretion, when calculated on the basis of weight, was practically the same in four out of five animals. There was no significant difference in the twenty-four-hour amount, the sulphate animals giving 1.55 mg., and the chloride animals 1.44 mg. In a fourth series with rabbits observations were made at twenty-minute intervals over four hours, and two drug doses two hours apart were administered. These animals yielded results which were less uniform than the rats, but they were individually consistent. The average maximum yield with the rabbits during the first two-hour period was 13.4 mg. of uric acid per 100 c.c. (the lowest being 6.3, and the highest 18.2), as compared with an average of 8.4 mg. per 100 c.c. obtained at the end of the first twenty-minute period (the lowest, 4; the highest, 11). For the second two-hour period the average maximum yield was 12.7 mg. of uric acid per 100 c.c. (the lowest, 8.5; the highest 15.4).

EXPERIMENTAL

Drugs.—Theophylline calcium salicylate (phyllicin), theobromine calcium salicylate (theocalcin), theophylline sodium acetate (theocin soluble), theobromine sodium salicylate (diuretin), theophylline, and theobromine are the drugs studied in this investigation.

Methods.—The data were from 149 adult rabbits which were kept on a complete and balanced diet determined for the individual animal. The daily water-intake was carefully controlled. The animals were housed in individual metabolic cages, and new animals were used for each dose series. The animals were observed first over a period of days to establish whether they were in a state of metabolic equilibrium. The drugs, dissolved in or mixed with definite volumes of water, were administered by stomach tube three times a day (morning, noon, and evening) over a period of four days. The observations

TABLE I

DIURETIC EFFECTS OF CERTAIN XANTHINES ON RABBITS. UNIFORM FLUID INTAKE: 150 C.C. DAILY. DRUG GIVEN ON FIFTH THROUGH EIGHTH DAYS												
ANIMAL	DRUG AND DOSE GM. T.I.D.	DAY	TOTAL DAILY OUTPUT	COLOR TURB. REACT.	S/G	TOTAL SOLIDS GM. P. C.C.	URIC ACID GM. P. L.	UREA MG. P. C.C.	ALBUM. GM. P. L.	SUGAR PER CENT	MICRO- SCOPIC	OTHER AB- NOR- MALITIES
7	Normal premed- ication	1	55	*y/s/al	1.013	0.030	0.17	0.024	0.25	neg.	neg.	neg.
		2	60	y/m/al	1.014	0.033	0.17	0.016	0.25	neg.	neg.	neg.
		3	88	y/m/al	1.015	0.035	0.18	0.014	0.25	neg.	neg.	neg.
		4	41	y/m/al	1.012	0.027	0.19	0.024	0.25	neg.	neg.	neg.
	Averages		61	y/m/al	1.014	0.031	0.18	0.019	0.25	neg.	neg.	neg.
		5	143	a/s/ac	1.014	0.033	0.17	0.018	0.2	neg.	neg.	neg.
		6	70	a/s/al	1.025	0.057	0.25	0.012	tr.	neg.	neg.	neg.
		7	70	y/m/al	1.018	0.042	0.25	0.014	tr.	neg.	neg.	neg.
	Averages	8	85	y/m/al	1.014	0.033	0.24	0.014	0.2	neg.	neg.	neg.
			92	a y/sm/al	1.018	0.041	0.23	0.017	0.1	neg.	neg.	neg.
		9	70	y/s/ac	1.016	0.037	0.17	0.015	0.1	neg.	neg.	neg.
		10	85	y/s/al	1.014	0.033	0.14	0.012	0.3	neg.	neg.	neg.
Postmedication	11	55	y/m/al	1.012	0.027	0.12	0.012	tr.	neg.	neg.	neg.	
	12	59	y/m/al	1.012	0.027	0.12	0.010	0.2	neg.	neg.	neg.	
		67	y/sm/al	1.013	0.030	0.14	0.012	0.15	neg.	neg.	neg.	
	Averages											

*y. yellow, s. slight, al. alkaline, a. amber, m. moderate, ac. acid.

*y, yellow. s, slight. al, alkaline. a, amber. m, moderate. ac, acid.

were continued thereafter until the daily output of urine approached that of the premedication period. Twenty-four-hour specimens of urine were collected, and the daily observations included the total volume of urine voided, the determination of the physical characteristics of the urine, the specific gravity, reaction, total solids, uric acid, urea, albumin, sugar, and microscopic examination.

Specific gravities were determined by the pycnometer method. Total solids were estimated by the coefficient of Haeser, since the quantities of urine were limited. Uric acid was determined by the method of Morris and Macleod.⁵⁵ Urea was estimated by an adaptation of the method of Folin and Wu⁵⁶ for blood analyses. Albumin was tested qualitatively by Roberts' test.⁵⁷ and, in the event a positive reaction was obtained, Esbach's method⁶¹ was employed for quantitative determinations because of the limited amounts of urine. Sugar was tested qualitatively by Benedict's test.⁶² When positive, Benedict's method⁶³ was employed for quantitative determinations. Chlorides were estimated by Whitehorn's method.⁶⁴ Allantoin determinations were attempted by Wiechowski's method,⁶⁵ but the results were unsatisfactory because of the small amounts of urine and the dilution. Each specimen was centrifugalized and the sediment was examined microscopically.

Table I shows the type of daily records which were kept for each of the 149 animals used.

OBSERVATIONS

Series I. Theophylline Calcium Salicylate.—This drug, in doses of 10, 20, 40, 50, 60, 75, 100 and 150 mg. per kilo three times a day, was administered to a series of 37 animals. The results obtained (Table II) show that with rabbits 60 mg. doses are the most efficient, if measured by increased urine output and maximum twenty-four-hour output, and that 50 mg. doses are most efficient from the standpoint of increased uric acid elimination and the duration of increased diuresis. There was no evidence of local irritation until doses of 100 mg. or more were reached, when the animals began to manifest loss of ap-

TABLE II
EFFECTS OF VARIOUS DOSES OF PHYLICIN ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	27	75	18	125	—	—	7½ days	1, 3 dg.;* 2 p.
20	45	93	73	140	—	—	7 days	1, 4 dg.; 1, 2 p.
40	43	81	23	184	—	—	7 days	1, 4 dg.; 2 p.
50	74	140	79	395½	—	—	9 days	1, 2, 3 dg.; 2, 3 p.
60	106	188	86	233	—	—	7½ days	1 dg.
75	94	125	94	280	—	—	8 days	1, 3 dg.
100	105	176	68	537½	plus	—	8 days	1 dg.; 3 p.
150	-34 to +81	---	27	100	Four of seven animals died on the first or second drug day. Casts in urine: gastrointestinal irritation.			

* 1, 2, 3 dg. = 1st, 2nd, 3rd drug day throughout tables. 1, 2, 3 p. = 1st, 2nd, 3rd post-medication day. OP. = output.

petite and diarrhea, and increased albumin in the urine. In cases of death, autopsy disclosed gastroenteritis and renal irritation. No deaths occurred until doses of 150 mg. or more were administered.

Series II. Theobromine Calcium Salicylate.—Doses of 10, 30, 50, 75, 100, 150, and 400 mg. per kilo three times a day were administered to a series of 27 rabbits. In the case of this drug (Table III) 50 mg. doses appear to be the most

TABLE III
EFFECTS OF VARIOUS DOSES OF THEOCALCIN ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	61½	110	67	217½	—	—	7½ days	1, 2, 3 dg.
30	22	78½	44	180	—	—	6½ days	1 dg.; 2 p.
50	97	210	48½	187½	—	—	8½ days	1 dg.
75	30	82	19	134	—	—	7 days	1, 2, 3 dg.
100	58½	136	56½	227	plus	—	7 days	1 dg.
150	—14 to	---	54	131	—	plus	-----	-----
	plus 50	---			One animal died second drug day			
400	—10 to	---	39	39	---	plus	-----	-----
	plus 179½				All animals died second drug day			
Urinary casts and gastrointestinal irritation were observed in all animals that died								

efficient. Although 100 mg. doses produced the greatest increase in uric acid eliminated, irritation resulted from such quantities, and deaths occurred when doses of 150 mg. and over were given.

Series III. Theophylline Sodium Acetate.—Twenty animals were used with this drug which was administered in doses ranging from 10 mg. to 100 mg. per kilo three times a day. Thirty milligram doses (Table IV) produced the

TABLE IV
EFFECTS OF VARIOUS DOSES OF THEOCIN SOLUBLE ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	33	72½	48	154½	—	—	6½ days	1, 3 dg.; 1, 2 p.
30	49	88	58½	189	—	—	6½ days	1, 3 dg.; 2 p.
50	35	96	34	164	plus	—	5 days	1, 3 dg.
75	64	120	36	260	plus	—	7½ days	1, 3 dg.; 3, 4 p.
One animal died third drug day								
Two animals lost weight during drug period								
100	131	100	90	267	plus	—	6½ days	1 dg.; 2 p.
Two animals died during second drug day								
All animals that died showed casts in urine, and gastroenteritis								

most efficient results within safe limits. Evidence of irritation was obtained with doses of 50 mg. and over. No deaths occurred until doses of 75 mg. or more were given. In cases of death, autopsy disclosed evidence of irritation of the kidneys and the gastrointestinal tract.

Series IV. Theobromine Sodium Salicylate.—Doses of 10, 30, 50, 75, and 100 mg. per kilo were used with 20 animals. The most efficient dose (Table V) was 50 mg., if determined by the increased urine output and the increase in solids eliminated. Larger doses produced a greater elimination of uric acid,

TABLE V
EFFECTS OF VARIOUS DOSES OF DIURETIN ON RABBITS

DOSE MG. PER KIL. T.I.D.	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBUMIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	50	92	45	72	—	—	7 days	3, 4 dg.; 1, 6 p.
30	44	94	51	88½	—	—	8 days	1, 4 dg.; 2, 4 p.
50	57	97	52½	158	—	—	7½ days	3, 4 dg.; 1 p.
75	26	108	34½	215	—	—	7½ days	1 dg.; 2, 3 p.
100	46	115	53	262	—	—	8½ days	1 dg.; 3, 4 p.

but the increases in the volume of daily urine-output and of solids eliminated were less than those resulting from the 50 mg. doses. The amount of uric acid eliminated increased with the dose. There was no evidence of irritation, and no deaths occurred (maximum dose used was 100 mg.).

Series V. Thecophylline.—This drug was administered to 19 animals in doses ranging from 10 mg. to 75 mg. per kilo three times a day. The most efficient dose (Table VI) appears to be 50 mg. However, the results obtained with doses

TABLE VI
EFFECTS OF VARIOUS DOSES OF THEOPHYLLINE ON RABBITS

DOSE MG. PER KILO T.I.D.	AVER. % INCR 'D OP.	AVER. % INCR 'D MAX. OP.	AVER. % INCR 'D SOLIDS	AVER. % INCR 'D URIC A.	INCR 'D ALBUMIN	INCR 'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
10	53	146	37½	72	—	—	9½ days	2, 3 dg.; 2, 3 p.
20	36	78	33	117	—	—	6 days	1, 3 dg.; 2 p.
30	53½	118	18	152	—	—	7½ days	1, 3 dg.; 3 p.
50	99½	162	69	222½	—	—	7½ days	1, 2, 3 dg.; 3 p.
75	51	174	56½	192	Three out of four animals died; one on first postmedication day; the others on the second drug day. Casts in urine, and gastroenteritis.			

TABLE VII
EFFECTS OF VARIOUS DOSES OF THEOBROMINE ON RABBITS

[illegible]

of 10 mg. as compared with those resulting from the administration of 20, 30, and 75 mg. doses are interesting. The maximum dose (75 mg.) produced death in 3 out of 4 animals, the kidneys and the gastrointestinal tract showing the effects of irritation.

Series VI. Theobromine.—Graduated doses of this drug ranging from 10 mg. to 200 mg. were given to 26 animals. The most effective safe dose from the standpoints of increased maximum output of urine, increased solids, increased elimination of uric acid, and duration of diuresis, is 40 mg. (Table VII), although 20 mg. doses produced a greater increased daily urine output. Deaths occurred with doses of 75 mg. and above, and autopsy showed renal and gastrointestinal irritation in each fatal case.

DISCUSSION

From a consideration of the foregoing data it is evident that the xanthine derivatives included in this study are capable of producing a good diuresis in rabbits, inducing increased elimination of solids and of uric acid, and maintaining a diuretic action for from two to about five and a half days after the administration of the drug is stopped. It is obvious also that in the overdosage established during this study these agents are capable of producing gastroenteritis, nephritis, and subsequent death, but that there is a fair margin of safety. The increase observed in solids was due primarily to chlorides. In general, within safe limits, it appears that when the urine output is increased, the rate of chloride excretion is accelerated until an optimum plateau is reached. Veil and Spiro⁶⁶ found that theophylline causes a prolonged fall of serum chloride even in animals whose kidneys have been removed. They conclude that theophylline affects not only the kidneys but also other parts of the vasculature, promoting the passage of chlorides from the blood into the urine and tissues.

Urine Output.—From the standpoint of increase in daily urine output theobromine calcium salicylate (61½%) and theobromine (68 per cent) produced the greater increases in dosage of 10 mg. (see Tables VIII to XII). Theophylline calcium salicylate (45 per cent) in dosage of 20 mg. produced a greater increase in diuresis than 30 mg. doses of theobromine calcium salicylate (22 per cent) and theobromine sodium salicylate (44 per cent), and 20 mg. doses of theophylline (36 per cent); although theobromine (72 per cent) in 20 mg. dosage, and theophylline sodium acetate (49 per cent), and theophylline (53½

TABLE VIII
EFFECTS OF 10 MG. DOSES ON RABBITS

DRUG	AVER. % INCR 'D OP.	AVER. % INCR 'D MAX. OP.	AVER. % INCR 'D SOLIDS	AVER. % INCR 'D URIC A.	INCR 'D ALBU- MIN	INCR 'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin	27	75	18	125	—	—	7½ days	1, 3 dg.; 2 p.
Theocalcin	61½	110	67	217½	—	—	7½ days	1, 2, 3 dg.
Theocin Sol.	33	72½	48	154½	—	—	6½ days	1, 3 dg.; 1, 2 p.
Diuretin	50	92	45	72	—	—	7 days	3, 4 dg.; 1, 6 p.
Theophylline	53	146	37½	72	—	—	9½ days	2, 3 dg.; 2, 3 p.
Theobromine	68	123	19	92½	—	—	8½ days	2, 3 dg.; 3, 4 p.

TABLE IX
EFFECTS OF 20 AND 30 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBU- MIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (20)	45	93	73	140	—	—	7 days	1, 4 dg.; 1, 2 p.
Theocalcin (30)	22	78½	44	180	—	—	6½ days	1 dg.; 2 p.
Theocin S (30)	49	88	58½	189	—	—	6½ days	1, 3 dg.; 2 p.
Diuretin (30)	44	94	51	88½	—	—	8 days	1, 4 dg.; 2, 4 p.
Theophylline (20)	36	78	33	117	—	—	6 days	1, 3 dg.; 2 p.
Theophylline (30)	53½	118	18	152	—	—	7½ days	1, 3 dg.; 3 p.
Theobromine (20)	72	130	32	130	—	—	8½ days	1, 3, 4 dg.; 1, 2, 4 p.

TABLE X
EFFECTS OF 40 AND 50 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBU- MIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (40)	43	81	23	184	—	—	7 days	1, 4 dg.; 2 p.
Phyllicin (50)	74	140	79	395½	—	—	9 days	1, 2, 3 dg.; 2, 3 p.
Theocalcin (50)	97	210	48½	187½	—	—	8½ days	1 dg.
Theocin S (50)	35	96	34	164	plus	—	5 days	1, 3 dg.
Diuretin (50)	57	97	52½	158	—	—	7½ days	3, 4 dg.; 1 p.
Theophylline (50)	99½	162	69	222½	—	—	7½ days	1, 2, 3 dg.; 3 p.
Theobromine (40)	55	130	65	162½	—	—	9½ days	1, 4 dg.; 1, 4 p.

TABLE XI
EFFECTS OF 60 AND 75 MG. DOSES ON RABBITS

DRUG	AVER. % INCR'D OP.	AVER. % INCR'D MAX. OP.	AVER. % INCR'D SOLIDS	AVER. % INCR'D URIC A.	INCR'D ALBU- MIN	INCR'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (60)	106	188	86	233	—	—	7½ days	1 dg.
Phyllicin (75)	94	125	94	280	—	—	8 days	1, 3 dg.
Theocalcin (75)	30	82	19	134	—	—	7 days	1, 2, 3 dg.
Theocin S (75)	64	120	36	260	plus	—	7½ days	1, 3 dg.; 3, 4 p.
One animal died third drug day. Two lost weight during drug period.								
Diuretin (75)	26	108	34½	215	—	—	7¾ days	1 dg.; 2, 3 p.
Theophylline (75)	51	174	56½	192	Three animals out of four died.	—	—	—
Theobromine (60)	28½	95	26	171	—	—	7¾ days	1, 3 dg.; 2, 3 p.
Theobromine (75)	43	73	25	199	—	—	8¾ days	2, 3, 4 dg.; 3, 4 p.
One animal died second drug day.								

per cent) in 30 mg. doses produced a greater diuretic action. In doses of 50 mg. theophylline (99½ per cent), theobromine calcium salicylate (97 per cent), and theophylline calcium salicylate (74 per cent) topped the list. However, the most striking results were observed with theophylline calcium salicylate in doses of 60 mg. (106 per cent), and of 75 mg. (94 per cent), the increases being from 1.7 to 3.7 times those obtained with 75 mg. doses of the

TABLE XII
EFFECTS OF 100, 150, 200 AND 400 MG. DOSES ON RABBITS

DRUG	AVER. % INCR 'D OP.	AVER. % INCR 'D MAX. OP.	AVER. % INCR 'D SOLIDS	AVER. % INCR 'D URIC A.	INCR 'D ALBU- MIN	INCR 'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (100)	105	176	68	537½	plus	-	8 days	1 dg.; 3 p.
Phyllicin (150)	-34 to +81	---	27	100	Four of	seven	animals died.	
Theocalcin (100)	58½	136	56½	227	plus	-	7 days	1 dg.
Theocalcin (150)	-14 to +50	---	54	131	-	plus	One animal died second drug day.	
Theocalcin (400)	-10 to +179½	---	39	39	---	plus	All animals died second drug day.	
Theocin S (100)	131	100	90	267	plus	-	6½ days	1 dg.; 2 p.
Diuretin (100)	46	115	53	262	-	-	8½ days	1 dg.; 3, 4 p.
Theobromine (100)	65	189½	31	135			Three out of four animals died during drug period.	
Theobromine (200)	97	203	134	208	All animals died third or fourth drug days.			

other five drugs, theobromine sodium salicylate (26 per cent) having produced the smallest increase in this dosage. Theophylline calcium salicylate (105 per cent) in doses of 100 mg. produced an increased urine output of 105 per cent as compared with 131 per cent in the case of theophylline sodium acetate, 65 per cent in the case of theobromine, 58½ per cent in the case of theobromine calcium salicylate, and 46 per cent in the case of theobromine sodium salicylate. However, deaths occurred in the cases of theophylline sodium acetate and theobromine; theobromine sodium salicylate alone in this dosage gave no indications of renal irritation. The greater efficacy of the calcium derivatives suggests the possibility that the calcium ion enhances the diuretic properties of the xanthine with which it is associated.

Uric Acid.—As shown in the introduction, the effect of the methylated xanthines on uric acid excretion has long been a subject of controversy. The data obtained in this study make it obvious that there is a definite increase in the uric acid excreted. The administration of uric acid or nucleins is followed by an increased excretion of uric acid which is more or less closely proportional to the amount of the agent administered. Since it has been established that demethylation does occur in the body, it seems not unlikely that a part of the ingested methylated xanthines may be completely demethylated, and that resulting products may be subsequently oxidized to uric acid. Thus Burian and Schur⁶⁷ found that the uric acid injected subcutaneously into man was excreted in the urine to the extent of approximately 50 per cent, these investigators believing that the other half was broken down into unidentified products. Likewise, when hypoxanthine was ingested, about 50 per cent of its nitrogen was accounted for in the immediate increase of uric acid excreted. Although the amount of uric acid eliminated, following the ingestion of these agents, increases in a roughly uniform manner with the increase in the dose of the drug, the calcium derivatives, theophylline calcium salicylate and theobromine calcium salicylate, are exceptions in that with these agents the doses

of greater diuretic efficacy (within safe limits), as measured by the increase in urine output, produce greater uric acid excretion than larger safe doses. The action of these derivatives is apparently specifically confined to uric acid and does not involve the other nonprotein nitrogenous constituents. It is not easy to explain the mechanism by which some diuretics increase the excretion of one constituent more than that of another. The effect may be on the force with which individual substances are held in the blood and tissues, or it may be a direct effect on the tubule epithelium influencing reabsorption to a different extent.

The percentages in the tables expressing increase in the uric acid excreted may be unintentionally self-exaggerating because of the small amounts of uric acid in the urine before medication. To illustrate: In the case of one rabbit which received 20 mg. doses of theophylline calcium salicylate, the daily output of uric acid during the premedication period averaged 11.7 mg. (or 5.85 mg. per kilo of body weight), and during the medication period the average daily output rose to 31.6 mg. (or 15.8 mg. per kilo of body weight), an increase of 19.9 mg. (or 9.9 mg. per kilo of body weight). This represents an increase of 170 per cent in uric acid, although the increase was but 9.9 mg. per kilo or 0.99 mg. per 100 gm. of body weight.

In 10 mg. doses theobromine calcium salicylate (217½ per cent), theophylline sodium acetate (154 per cent), and theophylline calcium salicylate (125 per cent) produced the greater increases in uric acid eliminated. In 20 mg. doses theophylline calcium salicylate (140 per cent) induced a greater increase than theobromine (130 per cent), and theophylline (117 per cent). In doses of 30 mg. theophylline sodium acetate (189 per cent), theobromine calcium salicylate (180 per cent), theophylline (152 per cent), and theophylline calcium salicylate (140 per cent) were more effective. In 40 mg. dosage theophylline calcium salicylate produced an increase of 184 per cent and theobromine 162½ per cent; while in doses of 50 mg. theophylline calcium salicylate (395 per cent) manifested more than twice the powers of the remainder of the group with the exception of theophylline itself (222½ per cent). In this dosage theobromine sodium salicylate produced the smallest increase (158 per cent) in uric acid eliminated.

In 60 mg. doses theophylline calcium salicylate (233 per cent) effected a greater increase in uric acid elimination than theobromine (151 per cent); and in doses of 75 mg. theophylline calcium salicylate (280 per cent) again topped the list.

When the limits of safety of this group of agents as a whole were reached (doses of 100 mg. and over), theophylline calcium salicylate (537½ per cent) still exceeded all other members of the group in increasing uric acid elimination, although theobromine sodium salicylate (262 per cent) was the only member of the group which in 100 mg. doses manifested no symptoms of poisoning. However, the administration of theobromine (135 per cent) itself in dosage of 100 mg. produced death in three out of four animals.

Duration of Diuretic Action.—In 10 mg. doses theophylline manifested activity over a period of nine and one-half days; theobromine, eight and one-half

days; theophylline calcium salicylate, and theobromine calcium salicylate, seven and one-half days; theophylline sodium acetate, six and one-half days; and theobromine sodium salicylate, six days. In doses of 20 mg. and 30 mg. theobromine (eight and one-half days), theobromine sodium salicylate (eight days), theophylline (seven and two-thirds days), and theophylline calcium salicylate (seven days) produced the more prolonged action.

When doses of 40 mg. and 50 mg. were ingested, theobromine (nine and one-third days), theophylline calcium salicylate (nine days), and theobromine calcium salicylate (eight and one-half days) were the more effective members of the group.

In 60 mg. and in 75 mg. doses theophylline calcium salicylate (seven and one-half and eight and two-third days) and theobromine (seven and three-fourths and eight days) were approximately equally effective, although with the larger dosage one theobromine animal died on the second drug day.

All members of the group are prompt in action, increased diuresis starting in practically all cases on the first drug day.

Toxicity.—Theobromine sodium salicylate is the only member of the group which manifested, in the doses employed, no toxicity. However, it should be noted that in this study this drug was not administered in doses larger than 100 mg., and that theobromine itself in doses of 75 mg. produced death in one out of four animals, while with doses of 200 mg. the mortality was 100 per cent by the third or fourth drug days.

The limit of safety is reached apparently with theobromine, theobromine calcium salicylate, and theophylline when these agents are given in doses of 75 mg. per kilo three times a day. In the cases of theobromine calcium salicylate and theophylline calcium salicylate 150 mg. doses seem to be definitely toxic, although in 100 mg. doses, while showing no fatalities, both drugs produced a definite increase in the albumin in the urine.

Autopsies disclosed positive evidences of both renal and gastrointestinal irritation in all animals that died.

Relative Efficacy.—If the increase in urine output, the increase in solids eliminated, the increase in the elimination of uric acid, safety, and the duration of diuretic action, are taken into consideration (see Table XIII), theophylline calcium salicylate, in doses of from 50 to 60 mg. per kilo three times a day, is most efficacious, and theophylline itself in doses of 50 mg. stands second among the remainder of these xanthine derivatives so far as rabbits are concerned. Theobromine calcium salicylate in doses of 50 mg. stands third, close in efficacy to theophylline.

The results of these studies confirm the statements of Underhill and Pack³ that theophylline and theobromine are powerful diuretics in rabbits (as well as in man). The consensus of opinion in the literature that (1) theobromine is more reliable than theophylline; (2) that theophylline is a more prompt diuretic but shorter in action and more irritating to the kidneys and the gastrointestinal tract; (3) and that both of these xanthine derivatives are capable of producing irritation of the kidneys and the gastrointestinal tract, agrees only in part with the findings of this investigation. These studies disclosed

TABLE XIII
EFFECTS OF THE MORE EFFICACIOUS DOSES ON RABBITS

DRUG AND MG. PER KILO T.I.D.	AVER. % INCR 'D OP.	AVER. % INCR 'D MAX. OP.	AVER. % INCR 'D SOLIDS	AVER. % INCR 'D URIC A.	INCR 'D ALBU- MIN	INCR 'D SUGAR	DURATION DIURESIS THROUGH	DAY(S) MAXIM. OP.
Phyllicin (60)	106	188	86	233	--	--	7 $\frac{1}{2}$ days	1 dg.
Theophylline (50)	99 $\frac{1}{2}$	162	69	222 $\frac{1}{2}$	--	--	7 $\frac{2}{3}$ days	1, 2, 3 dg.; 3 p.
Theocalcin (50)	97	210	48 $\frac{1}{2}$	187 $\frac{1}{2}$	--	--	8 $\frac{1}{2}$ days	1 dg.
Phyllicin (50)	74	140	79	395 $\frac{1}{2}$	--	--	9 days	1, 2, 3 dg.; 2, 3 p.
Theobromine (20)	72	130	32	130	--	--	8 $\frac{1}{2}$ days	1, 3, 4 dg.; 1, 2, 4 p.
Diuretin (50)	57	97	52 $\frac{1}{2}$	158	--	--	7 $\frac{1}{2}$ days	3, 4 dg.; 1 p.
Theobromine (40)	55	130	65	162 $\frac{1}{2}$	--	--	9 $\frac{1}{3}$ days	1, 4 dg.; 1, 4 p.
Theocin S (30)	49	88	58 $\frac{1}{2}$	189	--	--	6 $\frac{1}{2}$ days	1, 3 dg.; 2 p.

TABLE XIV
TOXIC DOSES OF XANTHINE DIURETICS ON RABBITS

DRUG AND MG. PER KILO T.I.D.	AVER. % INCR 'D OP.	AVER. % INCR 'D MAX. OP.	AVER. % INCR 'D SOLIDS	AVER. % INCR 'D URIC A.	*MORTALITY
Theocalcin (150)	-14 to +50	--	54	131	1 out of 4 animals died on 2nd medication day
Phyllicin (150)	-34 to +81	--	27	100	4 out of 7 animals died on 1st or 2nd med. day
Diuretin	No deaths or evidences of renal or gastrointestinal irritation with doses up to and including 100 mg. Larger doses of this drug were not given.				
Theocin S (75)	64	120	36	260	1 out of 4 animals died on 3rd med. day. (NOTE: 3 out of 4 died following 100 mg. doses.)
Theobromine (75)	43	73	25	199	1 out of 4 animals died on 2nd med. day. (NOTE: 3 out of 4 died following 100 mg. doses.)
Theophylline (75)	51	174	56 $\frac{1}{2}$	192	3 out of 4 animals died; one on 1st post- med. day, two on 2nd med. day.

*Autopsies disclosed definite evidence of renal and gastrointestinal irritation.

the facts that in rabbits (1) theobromine and theophylline and their salts are equally reliable diuretics; (2) that in both of these groups diuresis began promptly within approximately the same time (usually the first medication day), and, in the more efficacious doses, was practically of equal duration; and (3) that renal and gastrointestinal irritation is produced by practically the same dosage of the members of each of these groups (excepting "diuretin," which was not administered in doses larger than 100 mg.).

In clinical doses Stewart²³ found that theobromine calcium salicylate produced less irritation than theobromine sodium salicylate. The findings of this study show that in rabbits with doses up to and including 100 mg. theobromine sodium salicylate gave no indications of irritation, whereas theobromine calcium salicylate in doses of 100 mg. and over produced an increase in urine albumin.

Vieth's and Leube's¹ observation that calcium salts of the xanthines apparently influence diuresis in rabbits is confirmed by the results obtained in these studies.

The conclusion of Emerson¹⁸ that there is no increase in albumin after theobromine is contradicted by these findings, since theobromine in 75 mg. doses and theobromine calcium salicylate in 150 mg. doses both gave definite evidence of an increase in urinary albumin.

The observations of Bodzynski,²⁰ Gottlieb,³⁰ Krüger,²¹ Schmidt,³² Hess,³³ Schmoll,³⁵ Mendel and Wardell,³⁰ and others that there is an increase in uric acid following the ingestion of theophylline and theobromine are confirmed by this work. The conclusion of Morris and Rees²⁷ that the output of uric acid is affected by the volume of urine more than any other factor does not agree with the results obtained in these studies.

The relative toxicity of the salts as compared with the free xanthines appears to be about directly proportional to the amount of the xanthine contained therein, with the exception of theophylline sodium acetate which is as toxic as theophylline.

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EMBOLISM AND THROMBOSIS OF LARGE BRANCHES OF THE PULMONARY ARTERY IN HEART DISEASE*

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OBSTRUCTION of the pulmonary artery, like that of other vessels may occur as a result of thrombosis or embolism. The two processes having so much in common, and often so difficult to differentiate are usually considered together in discussions of this subject. Although the literature abounds with general studies on thrombosis and embolism, the relationship between pulmonary obstruction and heart disease has been somewhat neglected.

The study of the mechanism of thrombosis has been vigorously pursued and the definite answer as to how and why intravascular clotting occurs is still to be forthcoming. John Hunter¹ believed that infection of the vessel wall was responsible for thrombosis. Later, Virchow² brought forth a noninfectious process as the cause, namely, a sluggish circulation, which with other factors predisposed to the clotting. He applied the term "marantic" to thrombi of such origin. In a study of 1,000 cases of thrombosis and embolism, Rosenthal³ found the incidence highest in individuals over forty-one years of age in whom heart disease was present. The inference would be that stasis of the blood resulting from a failing myocardium is responsible for thrombus formation. Experimental work, however, has shown that stasis alone, or in association with endothelial injury will not result in thrombus formation. It appears, therefore, that

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changes in the blood itself, physical, chemical and colloidal changes, are necessary in order that thrombus formation take place.

Emboli on the other hand are usually pieces of clot which have broken off. There are in general two types of pulmonary emboli,⁴ one in which a large branch of the pulmonary artery is occluded, and the other in which smaller branches are occluded to produce either local disturbances or infarction. A large embolus may pass through the heart and lodge in the trunk of the pulmonary artery.

McCartney⁵ in his review of 73 cases of pulmonary embolism has included 16 cases of so-called medical embolism, embolism occurring in nonsurgical cases. Of these 16 cases, 6 were associated with cardiac disease. From this data it may be concluded that about 8 per cent of emboli occur in cardiac disease. All of McCartney's heart cases suffered from hypertensive disease.

In this connection the work of Haggart and Walker⁶ is of interest. Studying the effect of quantitative occlusion of the pulmonary artery they found that until from 52 per cent to 66 per cent of the pulmonary circulation is cut off, there is no significant variation in the circulatory condition of the animal. The point at which failure occurs is sharply defined, since beyond this end-point a circulatory collapse is precipitated by minute increases in pulmonary arterial obstruction, but if this is not applied, no untoward change results. This experimental evidence coincides with the clinical findings of Means and Mallory,⁷ who report a case of occlusion of the main right branch of the pulmonary artery by an organized thrombus which had been present for a period of many days or even weeks.

The present study is based upon 25 cases of frank cardiac disease, appearing on the wards of Cleveland City Hospital. All cases at autopsy had occlusion of large main branches of the pulmonary arteries. In 6 instances the pathologic diagnosis was that of pulmonary embolus.

Of the 6 cases in which the diagnosis of embolism was made there were 5 males and 1 female. The youngest was thirty-six years of age and the oldest fifty-nine years, the average age being fifty-two years. Of these, 3 cases had mitral stenosis; 1 had hypertensive heart disease; 1 had syphilitic aortitis and general paresis; 1 had coronary disease. Strikingly enough, in all cases the cardiac mechanism was normal. This is of significance, because ordinarily emboli are thought of as occurring with arrhythmias, but here none of the cases show such abnormality. In only 1 of these patients was death of the type commonly associated with pulmonary emboli. This case, one of chronic mitral and aortic disease, was found moaning while asleep. Cyanosis and dyspnea developed and death was sudden. In all the other cases death could be attributed to additional factors, the pulmonary embolus being apparently of minor consequence and occurring as a terminal event. For example, 1 case had a cerebral embolus; 1 the wet brain of general paresis; 1 coronary thrombosis; and the other 2 chronic failure of from six to twelve months in duration.

We may conclude from these cases that the typical death attributed to pulmonary embolism, the chest pain, dyspnea, cyanosis and anxiety, may be

often absent when the condition occurs in the course of heart disease. It appears that pulmonary embolism occurs so late in heart disease that it represents a terminal event.

A possible source of the embolus was found in 3 cases. Pulmonary infarcts were present in 2 instances.

In the remaining 19 cases of this study, the pathologic diagnosis was that of thrombosis. The youngest of these was thirty-five years of age and the oldest seventy-three years. There were 8 females and 11 males.

In all but 3 of these, cardiac failure was present to an obvious degree. As near as can be determined, the average time of failure was three months. Thus about 85 per cent of the cases were associated with cardiac failure extending over long periods of time.

The 3 cases which had no failure are of interest. One had coronary disease with myomalacia; another cerebral necrosis with a thrombus straddling the bifurcation of the inferior vena cava; a third had a cerebral hemorrhage.

In the cases then, in which myocardial failure was absent, other conditions were present which were sufficient to produce lethal exitus. In all instances the pulmonary thrombosis may be considered as a condition coming at the termination of an otherwise fatal disease.

In the cases of thrombosis there is again a striking paucity of cardiac arrhythmia. Only 5 of the cases, approximately 25 per cent were fibrillating, and there was 1 case of premature beats. As in the process of embolism, auricular fibrillation is not a frequent companion of thrombus formation.

Various types of cardiac disease are represented in this series. Hypertensive heart disease occurred in 13 instances, about 65 per cent. Rheumatic heart disease appeared in 1 case. Syphilitic heart disease appeared 3 times. Coronary disease was present 2 times and chronic pulmonary emphysema once. When studied with respect to the number of these diseases admitted to the hospital, it would seem that hypertensive disease is represented a little more than anticipated and rheumatic heart disease a little less. Embolism is more often associated with rheumatic disease and thrombosis with hypertensive disease.

Pulmonary infarcts were present in 13 instances, 68 per cent of the cases of pulmonary thrombosis, a much higher incidence than in cases of pulmonary emboli.

The frequency of antemortem thrombi in the right side of the heart was studied. In 7 instances there were such thrombi present. In 4 cases the thrombi were in the auricles alone; in 1 case in the right ventricle alone; in 2 cases in both auricles and ventricles.

In 3 additional cases there were thrombi in the venous system. These thrombi were found in the inferior vena cava, the right iliac vein and the gastro-epiploic vein.

The following cases are representative of this series:

L. C., a negro male, forty years of age, was admitted Apr. 19, 1932, and died Aug. 20, 1932. He had been failing for four months. His heart was enlarged, gallop rhythm. Blood pressure was 160/100. Clinical diagnosis: diffuse vascular disease, and congestive cardiac failure. Anatomic diagnosis: nephrosclerosis, cardiac hypertrophy, thrombus right pulmonary artery, thrombosis right auricle and left ventricle, chronic passive congestion, and multiple pulmonary infarcts.

E. N., a negress, thirty-six years of age, was admitted Feb. 21, 1929, and died Mar. 13, 1929. She had had shortness of breath and swelling of ankles for two weeks. On examination she had Cheyne-Stokes respiration, left-sided hemiplegia, enlarged left ventricle, with systolic murmur at apex. Blood pressure was 114/64. Clinical diagnosis: cerebral accident, and either embolism hemorrhage or thrombosis. Anatomic diagnosis: mitral stenosis, mural thrombi, right auricle and ventricle, pulmonary embolus, patent ductus arteriosus, and encephalomalacia,* right.

One of the purposes of a study of this kind is to increase diagnostic skill as a result of the classification of disease. From the diagnostic standpoint there is nothing particularly distinctive about these cases. If, in a case of cardiac disease the typical symptoms of pulmonary embolism appear, the diagnosis is simple. From the cases studied, however, it appears that this is not likely to occur. In heart disease the pulmonary emboli are usually quiescent and give little manifestation of their presence. A similar statement can be made concerning thrombi. It can be said, none the less, that in a case of prolonged cardiac failure, with repeated indication of pulmonary infarction, embolism, or thrombosis of the pulmonary vessels may be anticipated. This is in accord with the opinion expressed by Brenner⁸ who in his report of 6 cases of thrombosis of large branches of the pulmonary artery, concludes that thrombosis usually occurs after severe congestive cardiac failure has set in and produces no evident additional symptoms though it probably hastens the end.

CONCLUSIONS

1. Twenty-five cases of cardiac disease with embolism or thrombosis of the larger pulmonary arteries are reviewed.
2. Hypertensive cardiac disease is most frequently associated with pulmonary thrombosis.
3. Rheumatic heart disease is most frequently associated with embolism of the pulmonary arteries.
4. Auricular fibrillation was present in only a few cases.
5. Diagnosis of these conditions usually must be made by inference.

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*This may have been due to a paradoxical embolus passing through the patent ductus arteriosus.

MULTIPLE TUMORS*†

REPORT OF A CASE

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WHILE the occurrence of multiple tumors in a single individual is of no definite scientific importance at this time, their presence, nevertheless, arouses interest and never fails to invite speculation as to the reason for their existence.

Puhr, in 1926, published a study of a series of 6,718 necropsies performed during the preceding twenty-one years at the Second Budapest Pathological Institute, finding 1,559 cases of tumors. Two hundred and nineteen of these, or 14.04 per cent of the tumor cases, showed multiple growths. Puhr concludes from his assembled data that the occurrence of multiple tumors in the same individual is common enough to indicate the existence of a "tumor diathesis" either congenital or acquired. It is to be noted that in this series only five of the cases of multiple newgrowths show more than one tumor of the malignant variety.

Egli, quoted by Murray, finds multiplicity of neoplasms in the same individual in 263, or 27.3 per cent, of 966 tumor cases, which were studied during a seven-year period at the Basel Pathological Institute. In twenty of these cases, multiple malignant growths were demonstrated.

Harlitz, quoted by Murray, found 16 cases of multiple malignant growths in 524 cancer autopsies seen during the period of time from 1900 to 1915. These sixteen cases thus represented roughly 3 per cent of the total number.

Murray states that thirty-two examples of multiple malignancy were found in a series of 4,219 examinations made at the Oslo Institute for the Norwegian Cancer Committee. Thus, multiple malignancy was encountered in 1 per cent of the cases.

From a study of the reports of these authors and of various statistical studies concerning the occurrence of malignant tumors, Murray concludes that the multiplicity of neoplasms is rare, particularly the coexistence of tumors of the malignant variety. He feels that there is probably inherent in one malignant tumor a tendency to inhibit the development of other malignant tumors.

Yamakawa has reported a case of coexistent gastric cancer, lung cancer, kidney adenoma and neurofibroma of the suprarenal. Luchsinger reports coexistent canceroid of the forehead, bronchial cancer, malignant hypernephroma and osteochondroma of the lungs. Seecef found coexistent carcinoma

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of the lung and carcinoma of the rectum, each producing typical metastases. Other cases of multiple tumors have been reported by Risak, Siebke, Ball and Reynolds, Perrotti, Jolkwer and others.

We wish to report a case of four different coexistent tumors found at

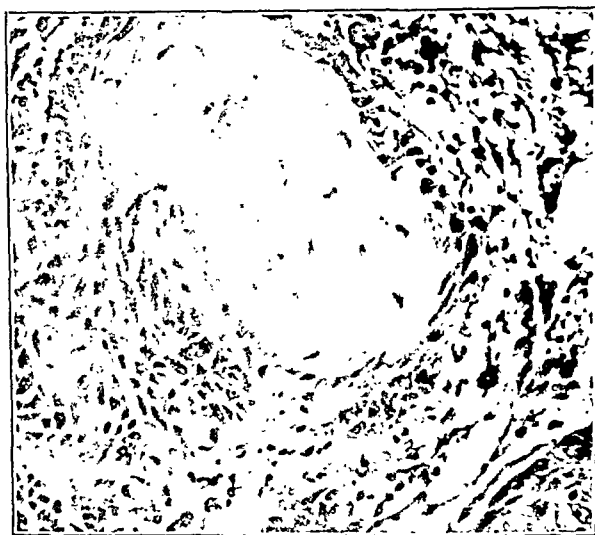


Fig. 1.—Sixteen mm. obj. oc. 15 \times camera 50 cm. \times 300 reduced. Penile tumor showing formation of epithelial whorl.

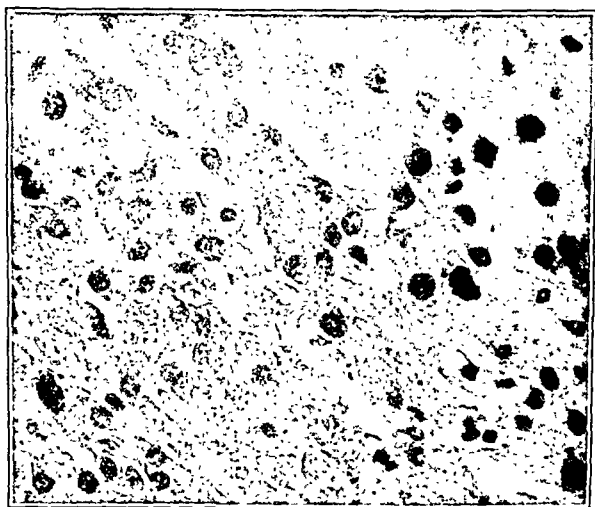


Fig. 2.—Eight mm. obj. oc. 15 \times camera 50 cm. \times 600 reduced. Hepatic tumor showing gradual transition from liver cords to the ill-defined tumor columns.

autopsy at the Edward Hines Jr. Hospital. This represents the only case showing multiple malignant newgrowths in a series of 364 autopsies.

CASE 1.—J. C., white male, aged sixty-two years, entered the hospital December, 1930, complaining of extreme weakness and loss of weight; a recurrence of penile growth which had occasioned a subtotal amputation about six months previously.

Family History.—Revealed nothing of interest.

Past History.—Patient denied venereal infection. In 1924 a rodent ulcer of the left jaw was removed surgically. There had been no recurrence of this tumor. In October, 1929, a lesion appeared on the foreskin of the penis which was later diagnosed as "cancer" and for which a subtotal amputation of the penis was performed in June, 1930. An ulcer on the

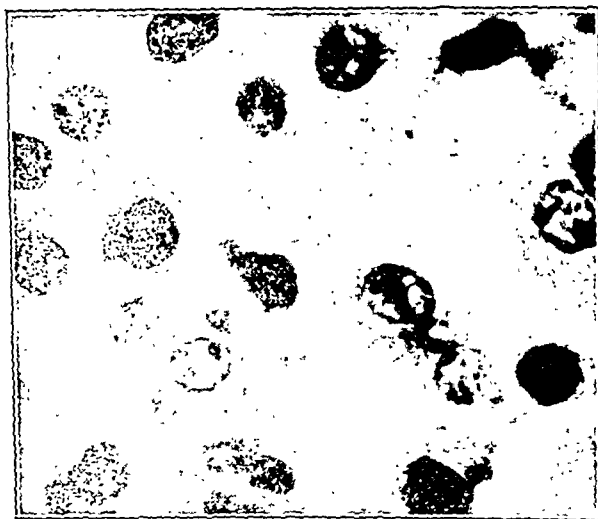


Fig. 3.—Four mm. obj. oc. 15 \times camera 50 cm. \times 1200 reduced. Liver tumor showing large cloudy cells forming ill-defined cords. Note the mass of pigment within space at center of cut.



Fig. 4.—Four mm. oc. 15 \times camera 50 cm. 1200 \times reduced. Liver tumor. Detail showing mitotic figure.

left cheek had been gradually enlarging over a period of ten years until at the present time it is 1.5 cm. in diameter.

Physical examination revealed an emaciated senile male who was in an extreme cachectic state. An ulcer of approximately 1.5 cm. in diameter was seen on the left cheek approximately 2 cm. below the outer canthus of the eye. A large cauliflowerlike growth involved the penoscrotal junction. The nodes of the right inguinal region were enlarged.

The other physical findings were irrelevant.

Clinical Course.—The penile tumor and regional lymph nodes were repeatedly subjected to irradiation. Despite the treatment, the condition of the patient became steadily worse and death resulted on Jan. 7, 1931.

Autopsy resulted in the following anatomic diagnoses: Epidermoid carcinoma penis

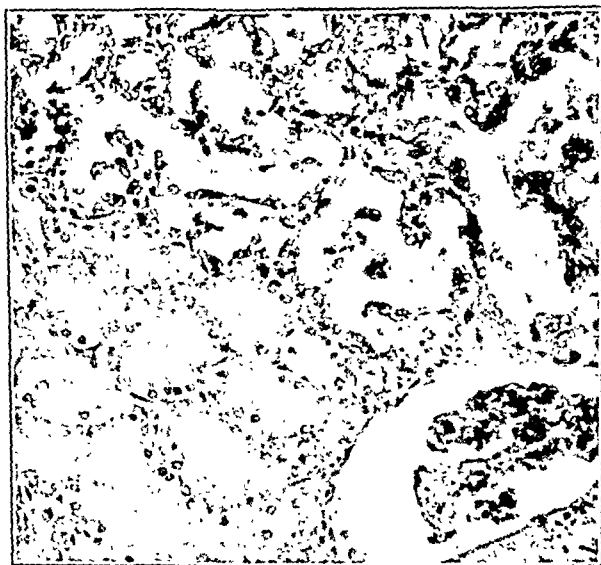


Fig. 5.—Sixteen mm. oc. 15 \times camera 50 cm. \times 200 reduced. Kidney tumor showing well-defined line of demarcation between renal tissue and atypical tubule formation of neoplasm.

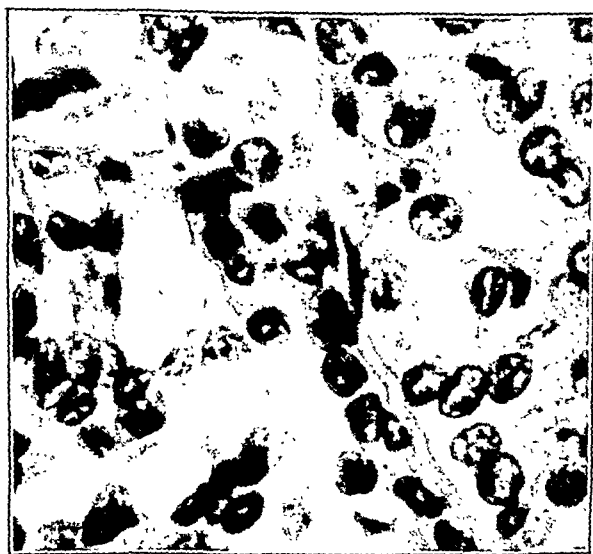


Fig. 6.—Eight mm. oc. 15 \times camera 50 cm. \times 600 reduced. Details of renal tumor. The atypical tubule formation is noted.

with metastases to regional lymph nodes; adenocarcinoma liver; adenoma kidney, right; carcinoma, basal cell type, face; bronchopneumonia; arteriosclerosis, advanced, severe; nephritis, interstitial, chronic, vascular type; myocardial degeneration; chronic passive congestion of liver; atrophy spleen, senile; hypertrophy prostate, adenomatous; hyperkeratosis, senile, left face; dermatitis, senile, legs.

Description of gross appearance of organs which proved on histologic examination to be the site of distinctly different neoplasms.

1. *Penis*.—The remnant of the penis was destroyed by a fungating cauliflowerlike growth. On section this tumor had a wartlike periphery, the inner portion being pearly white and of a cartilaginous consistency. The glands of both inguinal regions were dense and fibrosed but showed no gross evidence of tumor.

2. *Liver*.—A firm, grayish white nodule 3 cm. in diameter was found on the antero-lateral margin of the dome of the right lobe elevating the capsule somewhat. Cross-section through this mass revealed a homogeneous grayish white pearly surface of almost cartilaginous consistency. It was not encapsulated.

3. *Right Kidney*.—This organ weighed 140 gm. A nodule 2 cm. in diameter was located in the middle third of the cortex and bulged beneath the capsule. Section through the nodule revealed a yellowish white, smooth, homogeneous cut surface. This tissue was found to be of a firm consistency, but was not encapsulated although the edges were well defined.

4. *Skin Lesion Noted on Face*.—A shallow ulcer 1 cm. in diameter with elevated borders

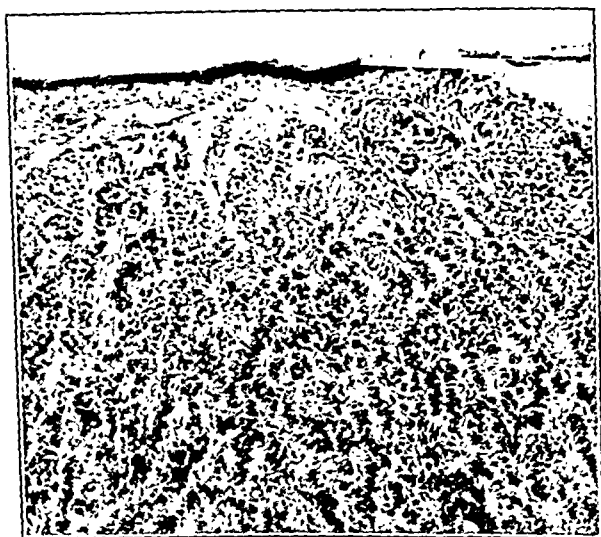


Fig. 7.—Sixteen mm. oc. 15 \times camera 50 cm. \times 300 reduced. Skin tumor. The epithelial invasion of the lower strata is noted.

of dense consistency was noted over the left molar region. The edge of the ulcer cavity was undermined. The base was filled with recent granulations. This lesion had the gross characteristics of a "rodent ulcer."

Histologic Examination of Penile Tumor.—Examination revealed a proliferation of squamous epithelium which invaded the deeper layers of tissue in long finger-like projections. The cells were well differentiated and contained much keratinization with the formation of epithelial "whorls." Mitotic figures were few in number.

Diagnosis.—Epidermoid carcinoma of penis.

Histologic Examination of Liver Tumor.—A regular epithelial cell proliferation forming ill-defined columns which closely resembled liver cords was noted. The cells were large, some being round and others elongated in shape. These cells showed a cloudy cytoplasm and a large open vesicular nucleus with a well-defined nucleolus. Mitoses were frequent. There was a definite well-marked fibrous stroma which was well vascularized. Indefinite channels, in many of which were small free masses of brown pigment, were formed between the cords of epithelial cells, probably in an ill-formed bile capillary. A gradual transition from the well-differentiated liver cell to the irregular atypical tumor cell existed without definite line of demarcation.

Diagnosis.—Adenocarcinoma of liver.

Histologic Examination of Kidney Tumor.—The epithelial proliferation tended to form columns and well-marked tubules of irregular size on well-vascularized stroma. The cells were large and oval with cloudy reticular cytoplasm. The nuclei were large and hyperchromatic and contained well-defined nucleoli. No mitotic figures were seen. The line of demarcation between the kidney parenchyma and tumor tubules was definite although the tumor was non-encapsulated. The latter lack the uniformity of true renal tubules both as to diameters and cytologic lining.

Diagnosis.—Adenoma of kidney.

Histologic Examination of Skin Lesion.—A well-marked proliferation of the lower layers of the epithelium infiltrated deeply into the submucosa. The cells were small, some round, and some oval with hyperchromatic nuclei. These cells formed masses with pseudocolumnar margins and closely resembled those seen in the basal layer of epidermis. No mitotic figures were noted.

Diagnosis.—Carcinoma, basal cell type.

SUMMARY

A case is reported in which at autopsy was demonstrated the simultaneous occurrence of four tumors, three malignant and one benign. Seven years previously the patient was operated upon for a rodent ulcer which did not recur.

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CHOOSING A SERUM TEST FOR SYPHILIS*†

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IN ADDITION to considerable laboratory experience with several of the numerous modifications of the Wassermann test, and with some of the so-called precipitation or flocculation tests, I have applied these serum tests to the diagnosis and management of syphilis in the clinic. It is, therefore, from a point of view which combines laboratory and clinical experience that I wish to consider the problem of choosing a serum test for syphilis.

The ideal or completely adequate test would be one which would give positive reactions in all cases of syphilis, and in no others. Thus, the first requirement for ideal adequacy would be that the test to be so sensitive as to detect the presence of syphilis from the beginning of the infection to the complete destruction of every *Treponema pallidum* in the body. According to present knowledge, no serum test can be expected to be entirely adequate in regard to this requirement because before the serum can give a positive reaction the *Treponema pallidum* must have resided in the body long enough to have evoked some sort of tissue response which directly or indirectly causes the detectable changes in the serum. Such changes rarely occur sooner than ten days after infection. Furthermore, serum tests are lacking in adequacy, in that, as yet no method has been devised which definitely indicates when the last treponeme has been destroyed. The second essential for an adequate serum test would be that it be specific in all cases. The occurrence of positive reactions with the serum of nonsyphilitic persons is obviously a serious fault.

From a practical standpoint there are certain other essentials for adequacy. The test must be dependable; its technic should be of such a nature that it can be accurately executed by the average laboratory worker, the results being consistent from day to day, and consistent with those obtained in other laboratories performing the same test. Furthermore, in addition to being sensitive, specific, and dependable, a method should be comparatively inexpensive. However accurate a new method is, it will not receive general recognition if the expense of performing the test is much greater than that of some of the excellent methods already in use. Finally, in order to survive, the method must be easy of execution, and easier still of interpretation.

Since it is inconceivable, from present knowledge, that any test could be completely adequate, one should be guided in the selection of a test for syphilis by its helpfulness to the physician.

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COMPLEMENT FIXATION TESTS

Since the Wassermann reaction is the oldest and most widely known test, I shall speak first of its inadequacies:

1. Lack of sensitiveness has taught able clinicians never to disregard unmistakable signs of syphilis merely on the strength of a negative Wassermann reaction. Failures of the Wassermann reaction are approximately 50 per cent in the primary stage of the disease, are few in the secondary stage, and vary between 40 and 60 per cent in the tertiary stage, depending upon treatment, duration, and so-called latency.

2. A positive Wassermann reaction is not always specific for syphilis, because certain acute respiratory infections, particularly pneumonia, and a few other conditions such as malaria and bacterial endocarditis, often cause positive reactions in individuals who are not syphilitic.

3. None of the many modifications of the Wassermann test is entirely dependable. Altogether too frequently there arises a situation in which a positive report is received from one laboratory, and a negative report from another, even though both laboratories are using the same Wassermann technic on separate samples of the same blood. Still more perplexing is such an occurrence as a report of a positive reaction in a given laboratory, followed very shortly, without any clinical reason, by a report of a negative reaction in the same laboratory.

In spite of these imperfections, we must not underestimate the immense contribution of the Wassermann reaction, for in doubling ability to detect the disease, it has, to date, I believe, done more to control syphilis than any other single factor.

FLOCCULATION TESTS

During the last ten years numerous tests have been devised for the purpose of increasing accuracy in the detection of syphilis and of more efficiently excluding it in differential diagnosis. Most of these new tests have made use of the principle, first stated in 1907 by Michaelis, and two years later affirmed by Jacobstahl, that the serum of a syphilitic person, when mixed with a suspension of suitably chosen lipoids, usually produces a change in the dispersion of the lipoids, leading to flocculation; the term "flocculation" is used rather than "precipitation" because flocculation describes a change rather than defines a mechanism, as yet not fully understood. It is not difficult to understand why so much progress has been made in the development of flocculation tests when one remembers that in order to effect a positive reaction with syphilitic serums, only the admixture of lipoids is necessary. On the other hand, the Wassermann reaction or any complement fixation test requires, in addition to the lipoids and the serum to be tested, three different reagents (amboceptor, complement, and sheep's cells), all three of which are biologic products whose respective potencies have never been controllable with certainty. It is now generally admitted that the flocculation tests are more dependable, and in my experience they have proved more specific than any of the complement fixation methods.

As a result of investigations of several flocculation methods in the laboratories under my direction, it has been possible to make the following comparable ratings in terms of adequacy, as expressed by sensitiveness and dependability. In a large group of cases studied it was found that the Eagle test possesses 72 per cent of the adequacy of the Hinton, the Kahn test 70 per cent, the Kline test from 71 to 80 per cent, and the Wassermann test from 38 to 46 per cent. The range of adequacy in the case of the Kline test has depended upon whether the antigen used was prepared in Kline's laboratory or in my own laboratory. With the former I obtained 71 per cent of adequacy, and with the latter I obtained 80 per cent of adequacy, as based on sensitiveness and dependability. With Kline's antigen which I prepared, however, the results suffered considerably in specificity, since there were several non-specific positive reactions. In the case of the Wassermann reaction, the range was greatly influenced by the type of cases, the sensitiveness being greater among the cases of syphilis from venereal disease clinics than among those of neurosyphilis from psychopathic hospitals.

It thus appears that in regard to sensitiveness and dependability there is little to choose between the Kline, the Kahn, and the Eagle tests. Accordingly, other factors must determine the choice. Each of the tests that I have mentioned presents certain difficulties. The Kahn test is admittedly difficult to read, although an unusual degree of accuracy in reading this test has been attained by those trained in Kahn's laboratory. The Kline test presents very few technical difficulties that are readily discernible; however, in our hands it has had its good and bad days. The difficulty with the Eagle test lies in its technic; in a laboratory in which a large number of tests are done, centrifuging each tube is difficult and impractical. The greatest difficulty with the Hinton test is that despite its simplicity it must be performed with exactness and precision for the best results. This can be obtained only by practice; in the hands of a serologic technician with no experience with the Hinton test, 80 to 90 per cent of adequacy can be obtained in less than a month, while the highest degree of accuracy in testing and reading usually requires experience of several months. Another practical disadvantage is the necessity of overnight incubation. These obstacles, however, I believe are outweighed by the greater sensitiveness and dependability of this test.

I feel strongly on the question of adequate sensitiveness of a test for syphilis; I have seen much suffering and great expense caused by a negative Wassermann reaction which, with absence of clinical signs, caused the physician to conclude that the disease was not present, when actually it existed in a communicable form. Thurmon's¹ figures show that during the first week after the appearance of the primary lesion, the Hinton reaction of the blood was negative in only 38 per cent of the cases studied; that in the second week of the primary lesion only 14 per cent were missed by the Hinton test, and that from the beginning to the end of the third week only 6 per cent of the cases remained negative. It is a well-known fact that in primary syphilis in which more than three weeks have elapsed since the beginning of the initial lesion, serum reactions are practically 100 per cent positive by any worth-while method.

In addition to the value which sensitiveness has for the early detection of syphilis, it has equal, if not greater, importance as an aid in the determination of cure. In the tests with which I have had experience, there has been none except the Hinton test which, in a large group of cases, was always positive whenever the spinal fluid showed a positive reaction by accepted tests.² This is of great importance to the general practitioner particularly, for it means that when the Hinton reaction of the blood is negative one will be unable, by lumbar puncture, to disclose laboratory evidence of neurosyphilis.

INTERPRETATION OF STATISTICS

In the determination of the adequacy of a test, statistics, which for the most part must serve as the guide, are often misleading. The two chief reasons for this are: (1) frequently the cases upon which figures are based are insufficiently studied clinically, and (2) the statistics are often erroneously prepared. Any such study, to be valid, must be based upon a complete as well as a careful clinical examination of each case by experts in syphilology. I would, for this reason, exclude all other than institutional cases in a statistical investigation of the adequacy of *any* serum test for syphilis. In referring to erroneously prepared statistics, I have in mind particularly those indicating the percentage of agreement between two methods. I can illustrate a frequently occurring fallacy in statistics by the following: Suppose a laboratory were to test 1,000 nonsyphilitic bloods, using two given methods, one of which was absolutely insensitive. The results would agree unless, perchance, one of the methods gave nonspecific reactions. If, using the same methods, tests are made upon 1,000 individuals, 10 of whom are definitely syphilitic, and 990 of whom are not, and one method finds the bloods of the 10 known syphilitic individuals positive, and the 990 normal persons negative its score will be 100 per cent. The method lacking sensitiveness finds not a single syphilitic's blood positive, though it finds the 990 negative; its true rating would be nil, yet, according to prevailing statistical methods, it would be computed as showing 99 per cent of agreement, a totally misleading figure. Suppose, however, that one has the bloods from 1,000 syphilitics. A given method finds 900 of these bloods positive; another test finds only 600 of these bloods positive. The accuracy of the first method is 90 per cent, whereas the accuracy of the other method is only 60 per cent. It is, therefore, manifest that in preparing statistics on the adequacy of a method, by comparison with another test, one must have, as a basic figure, the number of persons known to be infected. The efficacy of any method must be determined primarily on the basis of the number of actual syphilitic patients, and secondarily on a control group consisting of a sufficient number of nonsyphilitic persons to demonstrate that the test gives no nonspecific reactions, or, at most, only an occasional one.

FAITHFUL EXECUTION OF AN AUTHOR'S TECHNIC

Having chosen a method because of belief in its adequacy, as manifested by its sensitiveness, specificity, and dependability, it is incumbent upon the worker to execute each step of the test in the exact manner prescribed by the

author. There are several reasons for this caution, which might perhaps better be called "admonition." Before publication of his test, the author has carried on hundreds of experiments to perfect his technic. His directions and instructions are based on the technic he has found most successful, and no change should be made except on the basis of equal or more painstaking research. I recently read an abstract of an article on the Hinton test which stated that the sole advantage of the test was that it was easier to perform, but that it was no more sensitive than the Wassermann test. There was, however, a statement in the article that 0.1 c.c. and 0.3 c.c. of serum had been used in the Hinton test, instead of 0.3 c.c. and 0.5 c.c. of serum as directed. In my experience, fully 30 per cent of the specimens that give a well-marked characteristic reaction with 0.5 c.c. of serum are negative when only 0.3 c.c. is used. My selection of 0.3 c.c. and 0.5 c.c. was based on an examination of several thousands of bloods of persons who were investigated clinically as well as serologically.

Besides strict adherence to technical details, each worker must remember that as sensitiveness of a method increases there must be increasing precision in its execution. The Hinton test, which has proved, in my hands, more than twice as sensitive as the Wassermann test, is perceptibly affected by slight differences in the temperature of incubation, whereas the same temperature variations have had no discernible effect on the Wassermann test. In diluting antigen for the Wassermann test, I have not been able to discover any marked difference in the result when slight variations of the method have been made. In the Hinton test, however, in three laboratories under my direction, the slightest variations in diluting the indicator (I prefer to call it indicator rather than antigen) have caused inconsistencies in the results. Several months ago I designed a special flask³ for the purpose of diluting indicators, and as a consequence the three laboratories almost invariably obtained the same results when the same specimens of blood were tested.

I have recently simplified the technic of the Hinton test (third modification).⁴ This simplified method, which has been in operation in my laboratories for the past six months, is being maintained because it is superior in sensitiveness and specificity to my former methods. Its description, according to the statement of my students, sounds difficult and austere, but after they have actually performed the test their response is that it is very simple. The reason why the test sounds difficult is that I have made the directions as explicit as I could; each admonition represents a pitfall, the discovery of which has occupied days, weeks, months, or in some instances even years.

SUMMARY

The chief value of a serologic method of testing for syphilis lies in a high degree of sensitiveness, specificity, and dependability; the ease of execution and interpretation, as well as economy in cost, are secondary qualifications. Flocculation methods fulfill these requirements best. Of those now available, choice must be made on the basis of accurate, but not misleading; statistical

studies, rather than on simple comparisons with the Wassermann or any other test. Whatever method is chosen, best results will almost invariably follow from the closest adherence to the author's directions.

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STUDIES ON HEMOLYTIC STREPTOCOCCI ISOLATED FROM HEMORRHAGIC SMALLPOX*

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POSSIBLY no group of bacteria can claim more varied and more numerous types of pathogenic activity than the streptococci. Although these micro-organisms are easily recognized, because of their morphology, the possibilities of their unwarranted behavior are always a source of interest to the research worker in these fields.

The hemolytic streptococci are responsible for inflammatory and suppurative conditions which vary greatly in severity and effect. Such lesions range from trivial localized inflammations of transitory duration to those of great intensity, spreading rapidly to the tissue spaces by way of the blood. Exogenous infection, associated sometimes with epidemic spread, is a conspicuous feature of certain diseases due to the hemolytic streptococci, and virulent strains may produce sudden and fatal septicemia without any apparent lesion at the site of the initial infection. Following Jenner's¹ notable work on smallpox, there was a series of attempts to prove the bacterial origin of variola, and the literature on the contents of vesicles is large and embodies a variety of opinions. Many bacteria have been described in this connection, some of them new, others old and well-known forms. Among the researches in this field, we find the works of Garre,² Protopopoff,³ Waele and Sugg,⁴ Perkins and Pay,⁵ and Hines,⁶ dealing with the various phases of smallpox and their relations to the streptococci.

Because of these facts the present studies have been undertaken, not to prove the organism in question to be the etiologic factor, but rather to study its cultural, morphologic, serologic, and immunologic characteristics, and thus classify the different strains by their reactions to the several stimuli.

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CULTURAL HISTORY

In the course of a rather severe epidemic of smallpox which occurred in Detroit several years ago (1924), the opportunity presented itself to study, culturally, fatalities, the result of hemorrhagic smallpox. The epidemic was widespread and the individuals observed were from various parts of the city. Each patient entered the hospital as a confluent type and the hemorrhagic developments appeared from four to eight days after admittance. The strains when cultured on blood agar were hemolytic, and when examined macroscopically, the colonies were similar. In all, 9 strains were isolated and for purposes of identification have been designated as "Var.," while those forms from scarlet fever, erysipelas, and puerperal septicemia have been identified by number only.

All cultures isolated from sources of fatal hemorrhagic smallpox were from adults, ranging in age from twenty-eight to forty years, none of whom gave any history of vaccination. Pure cultures were obtained in every instance although this fact is not stressed as being of significance as an etiologic factor. Strains isolated from puerperal septicemia were obtained from the blood of patients who later died. Streptococci obtained from erysipelas were in one instance (02168), a blood culture from a fatal terminus, and in the other (02005) a culture from a skin lesion. Both of these strains show high cross agglutinating titer with the Birkhaug strains. Those streptococci isolated from authenticated instances of scarlet fever were from throat cultures and both strains produce specific toxin of a high titer.

In the isolation of streptococci one requisite is that the culture medium be fresh and that its composition be such that the organism in question will grow satisfactorily. A great variety of both fluid and solid mediums have been advocated by research workers, most of them making use of tissue extracts of brain, kidney, or spleen. Previous experiments in the isolation of streptococci led to the conclusion that veal broth and the brain liver media of Hibler gave most satisfactory results. The standard veal broth was prepared, containing the aqueous extractives of 500 gm. of lean chopped veal, 5 gm. of *c. p.* sodium chloride, and 10 gm. of Witte's peptone per liter. Dextrose in the amount of 0.2 per cent was added. The final reaction was adjusted to P_H 8.0 and the broth distributed into 500 c.c. flasks, each containing 200 c.c. The tissue medium of von Hibler⁷ as modified by Goss, Barbarin and Haines⁸ was also employed in making blood cultures.

The blood was drawn at the bedside under aseptic conditions from the median basilic vein and transferred immediately into culture medium. A quantity of 2 c.c. was added to the veal infusion bouillon and a like amount to the flasks containing the Hibler medium. The seeded flasks were taken at once to the laboratory and 1 c.c. of the diluted blood added to a mixture of 5 per cent sterile rabbit blood and 2 per cent agar, thoroughly agitated and poured into sterile Petri dishes. The resulting mixture represented a 1:100 dilution of the original blood. The plates, veal bouillon, and the Hibler medium were then incubated for twenty-four hours at 37° C. after which they were examined macroscopically.

In every instance a pure culture of *Streptococcus hemolyticus* was obtained from both the fluid and solid mediums. The blood plates never showed less than 400 and in several instances the number was as large as 900 organisms per c.c., computed on the basis of the original sample. Well isolated, typical single

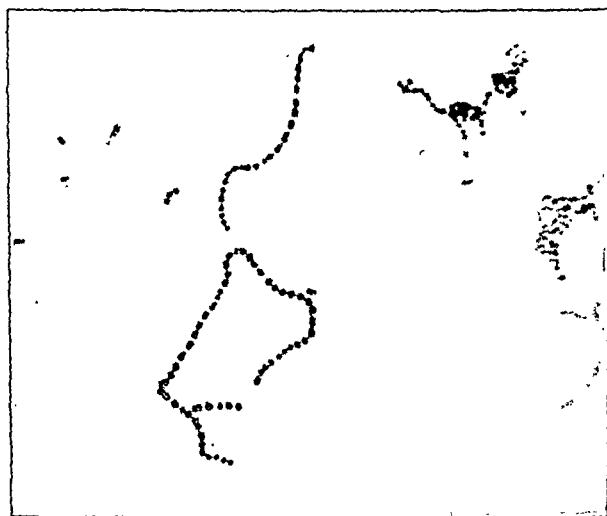


Fig. 1.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 8, twenty-four-hour bouillon culture. G. violet. $\times 1500$.

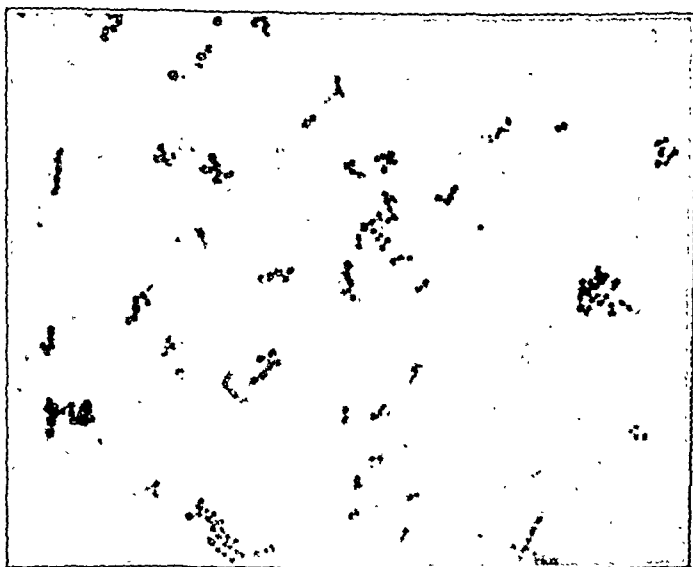


Fig. 2.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 8, twenty-four-hour blood agar culture. G. violet. $\times 1500$.

colonies, appearing on the blood plates, were selected and subcultures were made on blood agar slants, veal bouillon, and whole defibrinated sheep blood.

MORPHOLOGIC AND CULTURAL CHARACTERISTICS

The bacteriologic literature is crowded with classifications of organisms of every sort, based usually upon their metabolic activities, morphology, pathogenic

properties, manner of growth, and their ability to produce toxins, either soluble or insoluble. Numerous tests have been devised by thoughtful investigators to determine the type of organism under consideration. There occur, however, many criticisms of these classifications and as yet no general agreement has been reached relative to their respective values. In view of the various methods of classification and the apparent dissention among workers in this field as to their merits, it is not the intention of the author to attempt to classify the organisms

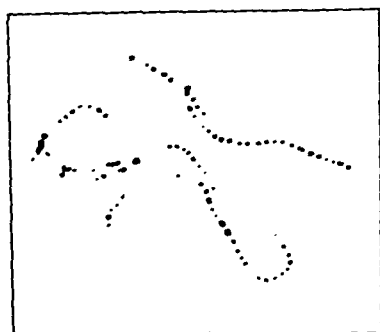


Fig. 3.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 3, twenty-four-hour bouillon culture. Negative strain. $\times 1500$.



Fig. 4.—Cultures from blood of individuals suffering from hemorrhagic smallpox. Both solid and liquid mediums. Strain Var. 3, blood smear from rabbit given 2 c.c. of a bouillon culture. Wright's stain. $\times 1500$.

in question by any certain concise methods, but to give a true picture of the reactions on different culture mediums according to the various workers.

The growth and morphology of each strain of *Streptococcus variolae* isolated, and cultures of streptococci from scarlet fever, erysipelas, and puerperal septicemia, were carefully noted, when cultured on infusion broth and blood agar. The data which are presented in Table I seem to indicate that there is but little variation between the strains.

TABLE I

MORPHOLOGIC AND CULTURAL CHARACTERISTICS OF STREPTOCOCCI ISOLATED FROM SMALLPOX, SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA

CULTURE	GROWTH		MORPHOLOGY		HEMOLYSIS	BILE SOL.
	BLOOD AGAR	BROTH	BLOOD AGAR	BROTH		
Var. 1	Small, smooth white colony	Clear, flaky sediment	Cocci in pairs and chains	Chains of 10 to 50	Wide zone	Negative
Var. 2	Small, smooth white colony	Clear, flaky sediment	Small cocci in pairs and chains	Chains of 6 to 50	Medium zone	Negative
Var. 3	Small, smooth white colony	Clear, flaky sediment	Small cocci in short chains	Chains of 10 to 50	Wide zone	Negative
Var. 5	Small, smooth blue white colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 10 to 50	Wide zone	Negative
Var. 6	Small, smooth blue white colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 8 to 50	Wide zone	Negative
Var. 7	Small, smooth white colony	Clear, flaky sediment	Small cocci in pairs and chains	Chains of 8 to 60	Wide zone	Negative
Var. 8	Small, smooth white colony	Clear, flaky sediment	Cocci in pairs and chains	Chains of 6 to 50	Wide zone	Negative
Var. 12	Small, smooth white colony	Clear, flaky sediment	Cocci in short chains	Chains of 10 to 50	Medium zone	Negative
Var. 14	Small, smooth opaque colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 6 to 40	Wide zone	Negative
Ery. 02168	Small, smooth white colony	Semi cloudy with sediment	Small cocci in short chains	Chains of 3 to 15	Narrow zone	Negative
Ery. 02005	Small, smooth white colony	Semi cloudy with sediment	Small cocci in short chains	Chains of 6 to 20	Narrow zone	Negative
Scar. 01730	Small, smooth colony	Clear, flaky sediment	Medium cocci in chains	Chains of 6 to 30	Wide zone	Negative
Scar. 01841	Small, smooth colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 6 to 22	Narrow zone	Negative
Puer. 02224	Small, smooth colony	Semi cloudy flaky sediment	Medium cocci in short chains	Chains of 6 to 30	Wide zone	Negative
Puer. 02087	Very small, smooth colony	Clear, flaky sediment	Medium cocci in short chains	Chains of 4 to 30	Wide zone	Negative

It appears from Table I that all the strains isolated from hemorrhagic smallpox were, in the main, very similar and indistinguishable culturally from other hemolytic streptococci, such as those from scarlet fever, puerperal septicemia, and erysipelas. They produce small, smooth colonies with hemolytic zones of varying diameters. Grown in veal bouillon they have a tendency to settle to the bottom in a flaky sediment.

The cells in strains 01841 and 01730, isolated from scarlet fever, were somewhat smaller than those isolated from the patients with variolae. In general, the strains from hemorrhagic smallpox produced a much wider zone of hemolysis than the others studied.

Figs. 1 to 4 show photomicrographs of 2 strains of streptococci isolated from smallpox cultured on solid medium, in bouillon, and also a direct blood smear from a rabbit.

FERMENTATION REACTIONS

Although the many authors who have carried out research on the fermentation reactions of the different streptococci are at variance in their opinions relative to the merit of this test, there is no doubt that characteristics thus established for the many strains of these organisms serve as a valuable aid in differentiating between the predominant types. Comparatively few have considered all criteria in classifying the strains studied by them. The Germans have given special attention to the morphology of the streptococci and their appearance on blood agar; the English have made an exhaustive study of the fermentation reactions of these organisms, while the Americans have divided their attention between one or the other set of characters. It is obvious that any research on the subject of the fermentation reactions of the streptococci must take into consideration certain essential precautions if reliable results are to be obtained. In these tests special care was exercised in the selection of the test substances in order that they be pure, sufficient time for the fermentation was allowed to elapse, and none of the test substances was injured by overheating.

All strains of the streptococci tested were first grown for 10 generations in veal infusion bouillon, P_H 8.0, and tested daily for purity. They were then transplanted for 3 generations on blood agar after which they were transferred to the carbohydrate medium. Double strength broth was made sugar-free in the usual manner, using *B. coli* as the fermenting agent. After complete fermentation the broth was filtered through a sterile Berkefeld filter, adjusted to P_H 8.0 and to every 200 c.c. of the sugar-free broth, 4 c.c. of Andrade's indicator, together with 4 c.c. of the test substance, was added. This was sterilized in flowing steam for twenty minutes on three successive days, cooled, and 200 c.c. of serum water (1 part horse serum to 300 parts of sterile distilled water) added under aseptic conditions. The finished carbohydrate serum broth was then tubed and incubated several days to insure sterility. Acid production caused by the fermentation of the test substance was noted by the color change and recorded. Table II shows the results of the fermentation action of the different strains of streptococci being studied.

It will be noted that with one exception all organisms isolated from smallpox are negative for lactose, while the control strains from erysipelas, scarlet

TABLE II

FERMENTATION REACTIONS OF THE STREPTOCOCCI FROM SMALLPOX, SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA

CULTURE	SOURCE	SACCHAROSE	DEXTRSE	MANNITE	LACTOSE	SALICIN	INULIN	LEVULOSE	D-MANNOSE	RAFFINOSE	XYLOSE	MALTOSE	CONTROL
Var. 1	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 2	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 3	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 5	Sm. Px.	+	+	+	+	+	-	+	+	-	-	+	-
Var. 6	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 7	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 8	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 12	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
Var. 14	Sm. Px.	+	+	-	-	+	-	+	+	-	-	+	-
01730	Scar.	+	+	-	+	+	-	+	+	-	-	+	-
01841	Scar.	+	+	-	+	+	-	+	+	-	-	+	-
02168	Ery.	+	+	-	+	+	-	+	+	-	-	+	-
02005	Ery.	+	+	-	+	+	-	+	+	-	-	+	-
02224	Puer.	+	+	-	+	+	-	+	+	-	-	+	-
02087	Puer.	+	+	-	+	+	-	+	+	-	-	+	-

fever, and puerperal septicemia all ferment lactose. This action, together with the action on the other test substances, would place the streptococci from smallpox under the head of *Streptococcus equi*, according to Holman's classification. The six comparative strains, would according to the same scheme, fall into that group, usually referred to as *Streptococcus pyogenes*. However, a careful review of numerous authors on the subject of *Streptococcus equi* shows such a variance of opinions that one is at a loss to know the true characteristics of the organism in question. In addition, each culture was planted in litmus milk and observed during a period of two weeks. The data are presented in Table III.

TABLE III

ACTION OF THE STREPTOCOCCI ON LITMUS MILK

CULTURE	SOURCE	ACIDITY	COAGULATION	REDUCTION
Var. 1	Sm. Px.	-	-	-
Var. 2	Sm. Px.	-	-	-
Var. 3	Sm. Px.	-	-	-
Var. 5	Sm. Px.	Faint, 14 hr.	+	-
Var. 6	Sm. Px.		-	-
Var. 7	Sm. Px.		-	-
Var. 8	Sm. Px.		-	-
Var. 12	Sm. Px.	-	-	-
Var. 14	Sm. Px.	-	-	-
02168	Ery.	+	-	-
02005	Ery.	+	-	-
01730	Scar.	+	-	-
01841	Scar.	+	-	-
02224	Puer.	+	-	-
02087	Puer.	+	-	-

From Table III we find that those strains of streptococci isolated from smallpox show no acid production in litmus milk while the comparative strains

all show marked acid production in twenty-four hours. This reaction according to Jones⁹ and Mathers,¹⁰ is characteristic of *Streptococcus equi* but is contradicted by the work of Besson¹¹ and Bergey.¹² Such opinions make a definite classification impossible. The fact that the strains of streptococci from smallpox vary among themselves and from the strains used as controls in their ability to ferment certain differential substances and their action on litmus milk, does indicate, however, that the strains in question are different from those usually classed as *Streptococcus pyogenes*.

VIRULENCE

The most significant property possessed by certain of the streptococci is that of dissolving red blood cells. The fact that this hemolytic action is confined to certain types of streptococci and not possessed by others serves as a method of classification and to some extent, bears a definite relation to the virulence of the organism. A review of the literature including the works of Rolly,¹³ Besson,¹¹ and McLeod,¹⁴ shows a difference of opinion, relative to the direct relation between hemolysin production and virulence.

In order that the virulence of the strains be at a maximum, they were serially transferred at twenty-four-hour intervals for 10 generations in 5 per cent horse serum broth, P_H 8.0. The culture representing the tenth generation was then injected into white mice which were subsequently killed. The heart blood was then transferred into tubes of serum broth and after twenty-four-hour incubation at 37° C., a portion of the bouillon was injected into a second

TABLE IV
VIRULENCE TESTS. HEMOLYTIC STREPTOCOCCI ISOLATED FROM SMALLPOX

CULTURE	MOUSE	AMOUNT	RESULT
Var. 1	1	1.0	Died, 48 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Lived
Var. 2	1	1.0	Lived
	2	0.1	Lived
	3	0.01	Lived
Var. 3	1	1.0	Died, 24 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Died, 72 hr.
	4	0.001	Lived
Var. 5	1	1.0	Lived
	2	0.1	Lived
	3	0.01	Lived
Var. 6	1	1.0	Died, 72 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Lived
Var. 7	1	1.0	Died, 48 hr.
	2	0.1	Lived
	3	0.01	Lived
Var. 8	1	1.0	Died, 24 hr.
	2	0.1	Died, 72 hr.
	3	0.01	Lived
Var. 12	1	1.0	Died, 24 hr.
	2	0.1	Died, 48 hr.
	3	0.01	Lived
Var. 14	1	1.0	Died, 48 hr.
	2	0.1	Died, 24 hr.
	3	0.01	Lived
	3	0.01	Lived

mouse. This technic was repeated until the virulence of each strain was at a maximum. Table IV contains the results of these experiments.

It would appear from Table IV that no strain of the streptococci isolated from smallpox is of high virulence for mice. Only 1 strain killed in a 1:100 dilution and the remaining strains killed only in a 1:10 dilution. It is to be noted that these strains are of a relatively low virulence when compared with those killing mice in dilutions of 1:100,000.

AGGLUTINATION EXPERIMENTS

The various immunity reactions so frequently employed for diagnostic purposes, such as agglutination, complement fixation, and bacteriotropine tests, give the most convincing evidence of biologic grouping. Many strains of bacteria, although indistinguishable by cultural methods, may be divided into separate groups. In the study of the hemolytic streptococci there seems from the beginning, some basis for an immunologic grouping of these organisms.

The immune scrums used in the serologic tests were obtained by the immunization of rabbits weighing approximately 2,000 gm. Previous experience, Ferry and Fisher,¹⁵ showed that individual rabbits immunized intravenously gave less variation and more constant results than those immunized by other methods. Each animal was given 3 intravenous injections of killed germs suspended in saline, preceded by one desensitizing injection, given subcutaneously. Five days after the last injection the rabbits were bled. The first, or desensitizing injection, given subcutaneously was 0.5 c.c. followed by 1 of 1 c.c. and 2 of 2 c.c. each, given intravenously three days apart. Two rabbits were treated with each of the 15 strains of streptococci being studied, after tests for agglutinins had been made against scarlet fever streptococcus 01730, puerpural septicemia 02087, erysipelas 02168, and smallpox Var. 3 and Var. 8. No rabbit was used whose serum showed any degree of agglutination in amounts less than 1:20 dilution. The suspensions to be used as antigens were grown in 100 c.c. quantities in a 0.1 per cent glucose broth, P_H 8.0, for twenty-four hours. The cultures were then centrifugalized and the sediment washed with saline to prevent the production of agglutinins to the protein constituents of the medium. The sediment was then taken up with normal saline solution containing 0.4 per cent tricesol and standardized in such a manner as to correspond to a suspension of 1,000,000,000 organisms per c.c. as this was found to be a safe dose for rabbits and compares closely to the count of broth cultures.

Agglutination tests may meet with difficulties on account of the tendency of streptococci to form clumps. The tendency of the streptococci isolated from smallpox to show spontaneous agglutination was overcome by using care in the preparation of the organisms to be used in the agglutination reaction. The broth was made from selected meat, and in place of the usual sodium chloride a sufficient quantity of dibasic sodium phosphate mixture was added to give the desired isotonicity and P_H . The organisms were removed from the culture medium by centrifugation and washed in broth prepared in the manner previously described. They were resuspended in the same type medium in approximately the concentration that was used in the agglutination reaction. The clumps were broken down by vigorous shaking and 0.5 per cent solution of

formalin added to kill the organisms. The suspensions of the controlling strains, while showing less tendency toward spontaneous agglutination, were prepared in the same manner as were the strains isolated from smallpox.

The serums employed in the tests were made up to the various concentrations by dilution with the same broth used for suspending the bacteria. Each reaction was controlled by a series of both normal serum, and suspension in the absence of serum. Equal quantities of dilute serum and bacterial suspensions were added to each tube and placed at 37° C. for eighteen hours. If this procedure is carefully followed there is less chance of nonspecific granulation occurring. If clumping develops in the broth control or in more than the first few normal serum controls, the test must be discarded.

Homologous and cross agglutinations were run with the 9 strains of smallpox streptococci and 2 each of scarlet fever, puerperal septicemia, and erysipelas streptococci. Table V gives the highest dilution of a specific serum showing definite agglutination against its homologous and heterologous strains. The 9 strains of streptococci from smallpox did not cross agglutinate to any appreciable extent with those from scarlet fever, puerperal septicemia, or erysipelas. There was, however, cross agglutination in high dilutions between the several strains from smallpox.

COMPLEMENT FIXATION REACTIONS

The principle of complement fixation has long been utilized in bacteriologic investigations, for determination, in the serum, of specific antibodies. Although a classification of streptococci on the basis of complement fixation has not proved as valuable as agglutination tests, it has been employed by many investigators to establish a natural classification of these organisms. In these studies, homologous and cross complement fixation tests were carried out with the same cultures as those used for agglutination, with three exceptions, due to the anti-complementary action of antigens prepared from Var. 5, Var. 8, and 02005.

Antigens were prepared in the following manner: Each culture was grown for twenty-four hours on 0.2 per cent dextrose sheep serum agar, P_H 8.0, in Roux flasks. The growth was washed off in 15 c.c. of normal saline solution and made up to 50 c.c. per flask. Each suspension was shaken for fifteen minutes and the remaining clumps allowed to settle for several hours. The supernatant fluid, containing the organisms was then decanted off, heated to 56° C. for one hour and killed with 0.5 per cent phenol. Stable antigens of all cultures, with the noted exceptions, were prepared by this method. They were nonhemolytic in quantities of 0.5 c.c. of a 1:5 dilution, nonanticomplementary in workable units, and comparatively high in antigenic value. The amount of antigen used was from 2 to 4 times the antigenic unit, usually 4 times, depending on the anti-complementary unit.

The tests were made by an adaptation of the Kolmer¹⁶ technic, the quantitative determinations being carried out with decreasing amounts of inactivated immune serum. Referring to Table VI one finds the results of the complement fixation studies. The average of the highest dilutions of the two rabbits immunized with a specific strain is taken as an index in each instance. A comparison of these results shows that they run about parallel with those of the agglutination tests.

TABLE V
 AGGLUTINATION RESULTS WITH STREPTOCOCCI ISOLATED FROM SMALLPOX, SCARLET FEVER, ERYSIPELAS, AND PUERPERAL SEPTICEMIA
 HIGHEST DILUTION OF SERUM SHOWING AGGLUTINATION AGAINST

ANTISERUM FOR	STREPTOCOCCI FROM SMALLPOX										SCARLET FEVER		ERYSIPELAS		PUERPERAL SEPTICEMIA	
	VAR. 1	VAR. 2	VAR. 3	VAR. 5	VAR. 6	VAR. 7	VAR. 8	VAR. 12	VAR. 14		01730	01841	02163	02005	02087	02224
Var. 1	3200	2400	600	2400	2800	2800	1400	2400	2000		20	20	200	20	400	200
Var. 2	1800	2800	400	1800	1800	1600	2400	1800	2000		20	20	20	20	20	200
Var. 3	2000	2400	1600	2000	1800	2800	2000	2400	2000		20	20	200	20	20	20
Var. 5	1600	1400	400	2300	1600	2400	1800	1600	1600		200	20	20	20	20	20
Var. 6	2400	2000	800	2400	2800	2800	1600	2100	2800		20	20	200	20	20	200
Var. 7	2000	1800	600	2400	2800	3200	2400	1800	2000		200	20	20	20	20	200
Var. 8	2400	2000	200	2400	3200	2800	2400	1600	2000		200	20	200	20	20	20
Var. 12	1800	1800	200	2000	1600	2400	2400	3200	2000		200	20	200	20	20	20
Var. 14	2000	2000	400	1600	1800	2800	2000	2800	3200		20	20	20	20	20	20
01730	20	200	200	20	20	20	200	200	20		2800	2400	1800	1800	1800	2000
01841	20	20	20	200	200	200	20	20	20		2400	2400	1800	1800	2000	1600
02163	20	200	20	20	20	20	200	200	20		2000	1000	2800	2000	2400	2400
02005	200	20	20	20	20	20	20	20	20		2400	2000	2800	2800	2400	2000
02087	20	20	20	20	200	20	20	20	20		1800	1000	1600	1800	2800	2400
02224	20	20	20	20	20	20	200	20	200		1400	1400	1600	1400	2400	2800

TABLE VI

COMPLEMENT FIXATION WITH THE STREPTOCOCCI
HIGHEST DILUTION OF SERUM SHOWING FIXATION AGAINST ANTIGENS OF

ANTISERUM FOR	STREPTOCOCCI FOR SMALLPOX							SCARLET FEVER		ERYSIP- ELAS	PUERPERAL SEPTICEMIA	
	VAR. 1	VAR. 2	VAR. 3	VAR. 6	VAR. 7	VAR. 12	VAR. 14	01730	01841	02168	02087	02224
Var. 1	800	600	80	1000	600	800	800	80	60	50	60	60
Var. 2	800	1000	80	600	500	500	600	80	80	60	60	60
Var. 3	600	600	200	500	800	600	800	60	80	60	60	60
Var. 5	500	400	80	600	1000	600	600	60	60	60	60	60
Var. 6	600	500	80	1000	800	600	800	60	60	50	50	60
Var. 7	800	600	100	800	2000	600	800	60	50	60	50	50
Var. 8	600	500	80	500	600	200	500	60	50	60	80	60
Var. 12	800	800	80	600	800	1000	800	80	100	60	60	80
Var. 14	800	800	80	800	800	400	600	60	80	60	60	80
01730	60	60	50	50	60	80	60	1000	1000	600	600	500
01841	60	80	60	50	80	60	50	800	800	600	600	800
02168	60	80	50	80	60	60	50	800	400	1000	600	800
02005	60	60	50	60	60	60	60	600	500	400	500	500
02087	60	80		60	60	60	80	600	600	600	1000	600
02224	60	30	60	60	80	60	60	600	800	800	800	1000

TOXIN PRODUCTION

During the last decade an exhaustive study of the streptococci and their soluble toxins has occupied a major portion of the time of many workers. In spite of extensive research upon the nature of the poisons produced by these microorganisms, our knowledge is still incomplete. The grave systemic symptoms often accompanying streptococcus lesions argue strongly for the production, by these organisms, of a powerful, diffusable, toxic substance. The toxins produced by the streptococci differ from most other true bacterial toxins in being thermostable. Many preparations will withstand boiling, and some may be boiled for an hour without entirely losing their capacity for skin reactions. Inasmuch as laboratory animals are not highly susceptible to the toxin, its standardization is limited at present to tests on susceptible human beings.

The question as to whether the streptococci isolated from hemorrhagic smallpox would produce a soluble toxin, thus presented itself as worthy of consideration. Was the fatal outcome of these cases due to a bacteremia, to a toxemia, or to a combination of the two? The toxin production was carried out on especially prepared medium. Veal infusion agar was made from carefully selected meat, and great care was used to remove all fat from the broth. One per cent Witte peptone, 0.85 per cent sodium chloride, c. p. were used and the broth titrated to P_H 7.8. The medium was placed in 200 c.c. quantities and seeded with the various strains of streptococci isolated from smallpox and with one strain each of scarlet fever, erysipelas, and puerperal septicemia. Incubation at 37° C. was carried on for six days when the cultures were filtered through sterile Berkefeld V filters and the filtrate preserved with 0.4 per cent phenol. Sterility determinations in bouillon and tests on guinea pigs were carried out to determine the safety of the product.

The cutaneous reactions were done at the Wayne County Training School, the individuals tested ranging in age from eight to sixteen years with an average

of twelve years. Both sexes were represented, although males predominated. Only those reactions which were positive to strongly positive were accepted and reactions under 1 cm. in diameter were regarded as negative.

TABLE VII
CUTANEOUS TESTS WITH TOXINS OF THE STREPTOCOCCI

	LEFT ARM				RIGHT ARM			
	VAR. 1 1:300	VAR. 3 1:300	VAR. 7 1:300	VAR. 8 1:300	01730 1:2000 SCAR.	02168 1:250 ERY.	02224 1:300 PUER.	CONTROL 1:250 HEATED
1. Y, R.	1.5 × 1.5		1 × 1	1 × 1		2 × 2	2 × 2	
2. R, E.		2 × 2	1 × 1			2 × 2		
3. J, K.							1 × 1	
4. P, M.	1.5 × 1.5	1 × 1	1 × 1	1 × 1		2 × 2	1.5 × 1.5	
5. S, K.						2 × 2	1 × 1	
6. S, M.						2 × 2	1.5 × 1.5	
7. B, D.			1 × 1	1 × 1	2 × 2	1 × 1	2 × 1.5	
8. A, H.		1.5 × 1.5	2 × 2	2 × 2	1 × 1	2 × 2	1.5 × 1.5	
9. M, M.						2.5 × 2.5	1.5 × 1.5	
10. L, R.	1 × 1	1.5 × 1.5	2 × 2	1 × 1	1.5 × 2	2.5 × 2	2.5 × 2	
11. S, E.								
12. K, L.				1 × 1		1 × 1	1 × 1	
13. Z, I.	1 × 1	1 × 1				1 × 1	1 × 1	1 × 1
14. A, S.	1 × 1	1 × 1	1 × 1	1 × 1		1.5 × 1.5	1 × 1	
15. D, W.						1 × 1	1 × 1	
16. A, A.		1 × 1	1.5 × 1.5	1 × 1		1.5 × 1.5	1.5 × 1.5	
17. A, G.	1 × 1		1 × 1	1 × 1	2 × 2	3 × 2	2 × 2	
18. J, H.		1 × 1				1 × 1	1 × 1	
19. J, E.	1 × 1	1 × 1		1 × 1		1 × 1	1 × 1	
20. P, A.	2 × 2	3 × 3	2 × 2	3 × 3		2 × 2	2 × 2	
21. F, C.	1 × 1	2 × 2	2 × 2	2 × 2	2 × 2	2 × 2		
22. H, A.								
23. F, J.								
24. D, R.	2 × 2	3 × 3	2 × 2	2 × 2	2.5 × 2	2 × 2		
25. D, T.		1 × 1	1 × 1		1 × 1	1 × 1		
26. D, G.		2 × 2	1 × 1	1 × 1	1 × 1			
27. H, O.		1 × 1		1 × 1	1 × 1	1 × 1		
28. B, H.								
29. S, E.		2 × 2			1 × 1			
30. M, J.		1 × 1	1 × 1	1 × 1	1 × 1	1.5 × 1.5		
31. S, L.	1.5 × 1.5	2 × 2	2 × 2	2 × 2	1 × 1	1 × 1		
32. K, N.	1.5 × 1.5	1.5 × 1	1.5 × 1	1 × 1	1.5 × 1.5	1.5 × 2		
33. S, J.					1 × 1			
34. K, G.		1 × 1	1 × 1	1 × 1	1 × 1	1 × 1		
35. M, J.								
36. M, M.					1.5 × 1.5	1.5 × 1.5		

The results of skin tests with the toxins of 8 strains of streptococci isolated from hemorrhagic smallpox and 1 each of the comparative strains, may be seen in Table VII. The control toxin in each instance was heated at 100° C. for thirty minutes in the autoclave. Varying dilutions of the filtrates were made, and subcutaneous injections, given on the forearm of the individuals tested, were read in twenty-four hours. Dilutions were made so that 1 c.c. of the original filtrate would contain 3,000 skin test doses. The toxins from 01730, scarlet fever, 02168 erysipelas, and 02224 puerperal septicemia, had all been previously neutralized with convalescent serum and also by horse antitoxin produced by injection of organisms from scarlet fever (Dick), erysipelas (Birkhaug), and puerperal septicemia (Birkhaug).

A study of the table would seem to indicate some relationship between the smallpox strains and those of the streptococci from erysipelas and puerperal septicemia. Out of a total of 79 individuals tested, only 14 gave reactions to the erysipelas and puerperal septicemia toxins and no reaction to the smallpox toxins. Individuals reacting to the puerperal septicemia and erysipelas toxins and one or more strains of the smallpox toxins numbered 45. The results would also indicate that the smallpox strains are closely related in their ability to produce soluble toxins.

In order to prove conclusively that the substance causing the reactions in the intradermal tests was a true toxin, an effort was made to produce an antitoxin. The streptococcus immune serum or antitoxin would thus neutralize its own toxin when mixtures of toxin and antitoxin were injected intradermally into an individual who was susceptible to the toxin. The immune serums were produced by repeated intravenous injections of undiluted filtrate into rabbits. The first injection was 3 c.c. of filtrate followed every third day by increasing amounts until a maximum of 10 c.c. was given. The animals were bled ten days after the last injection. A control serum was obtained from nonimmunized (normal) rabbits. Equal amounts of diluted filtrate and a 1:20 dilution of both immune and nonimmune serums were mixed and allowed to incubate for one hour at 37° C. Varying dilutions of the antitoxin were used in the test. Persons who were positive to the toxin of one strain of the streptococci from smallpox were selected and injected as shown in Table VIII.

Individual variations in the persons injected account for several unwarranted reactions. Two of the group were sensitive to the serum while two others reacted to the protein. The antitoxin in a dilution of 1:10 neutralized the toxins from Var. 7 and Var. 8 although a 1:20 dilution showed no neutralization in 2 instances.

SUMMARY

From the results of this work it would seem apparent that certain differences exist between the hemolytic streptococci isolated from blood cultures of fatal hemorrhagic smallpox and the pyogenic type of organism of scarlet fever, erysipelas, and puerperal septicemia.

Morphologically, the strains of streptococci from smallpox are similar to those isolated from scarlet fever, puerperal septicemia, and erysipelas.

Classification of the streptococci by means of their action on certain carbohydrates would place the organisms from smallpox and those from scarlet fever, erysipelas, and puerperal septicemia, in two distinct groups, based on the inability of the latter to ferment lactose.

The controlling strains each produced an acid reaction in litmus milk while the strains from smallpox were all negative.

The virulence for mice of organisms from smallpox is low, the usual fatal dose, after repeated passage, being about 0.1 c.c.

Agglutination tests with both homologous and heterologous strains would indicate a different serologic grouping between the organisms isolated from smallpox and the comparative strains.

Complement fixation results run about parallel to the agglutination tests and are apparently no more specific.

Streptococci isolated from smallpox produce a specific toxin which when injected into susceptible individuals gives rise to a cutaneous reaction.

The antitoxin produced in rabbits is specific as it neutralizes the toxin while normal serum has no effect.

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CHANGES IN BLOOD CONCENTRATION INCIDENT TO SHOCK*

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MONOGRAPHS on clinical pathology and microscopy contain no references to blood changes in traumatic shock or in circulatory failure of similar character developing in other conditions. Occasional references to such changes are found in clinical reports, but their significance and diagnostic importance have not been emphasized.

In 1893 Sherrington and Copeman¹ observed that animals, subjected to prolonged experimental operative procedures, presently showed increased respiratory and pulse rate, and decreased temperature and blood pressure. This was accompanied by the development of edema and by increased concentration of the blood. They suggested the relationship of these phenomena to surgical shock. It is of interest that these observations forecast by twenty-five years the accepted interpretation of shock, and that the essential features of it including increased blood concentration were noted. Cannon, Fraser and Hooper,² observing the development of shock in wounded soldiers, found a red cell count of 6 million or more and a corresponding increase in hemoglobin as prominent characteristics. Robertson and Bock³ corroborated those observations. Keith⁴

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showed that the increased blood concentration was due to loss of blood plasma and that it was accompanied by a marked decrease in total blood volume. These findings have been confirmed repeatedly. In shock produced experimentally an increased concentration of blood occurred so regularly that we used it as a criterion for shock in a study of the pathology of that condition.⁵

Dale, Laidlaw and Richards,⁶ by injections of histamine, reproduced experimentally in mammals all the physiologic manifestations of shock following trauma. They noted increase in the red cell count and hemoglobin as regular features. They found that histamine produces direct injury to capillaries, causing loss of tonus and increasing the permeability. Escape of plasma through damaged capillary walls resulted in concentration of the cellular elements of the blood. The visible circulatory changes produced by histamine are present in cases of traumatic shock and the mechanism of their production apparently is identical. In the final analysis the etiology of shock is loss of capillary tonus. Extensive capillary dilatation withdraws blood from currency as effectively as hemorrhage. The patient "is bled to death into his own capillaries."

Krogh⁷ of Denmark and Lewis⁸ of England have studied the physiology of the capillaries most extensively and intensively. Their results agree in showing that increased permeability and loss of tonus are produced by all types of damage to capillaries. Such damage may result from light, heat or cold, chemical agents both organic and inorganic, injurious bacterial products and particularly products of cellular injury, similar to histamine in action and in composition. Any of these agents appropriately applied, will produce the shock syndrome. One who, unaccustomed to light, exposes his skin surface to intense sunlight for a short time may develop shock in mild degree. Profound shock has resulted from overexposure to the sun. Similar results may follow overexposure to artificial light rich in ultraviolet rays. It is well known that shock regularly follows superficial burns, and that its degree is proportionate to the size of the burned area. The shock is profound and may be fatal if about one-third of the skin surface is burned or scalded. Many salts of heavy metals will produce shock if introduced into the circulating blood. Various organic compounds will produce similar disturbances. Sodium barbital, and closely related compounds used as sedatives, will produce shock when overdoses are taken. We have seen characteristic circulatory collapse develop in a dog following the intravenous injection of sodium barbital, 0.3 gm. per kg. of body weight. The blood pressure fell 94 mm. in three hours. The respirations and pulse rate became increased to twice the normal. The external parts became cold; there was 20 per cent increased concentration of the blood as shown by hemoglobin percentage and erythrocyte count. At postmortem examination all the changes which we have found to accompany shock were present. These, it should be emphasized, are identical to those observed by Dale, Laidlaw and Richards, following death from histamine (see Figs. 1 and 2). The abdominal and thoracic viscera had a deep rose-red color due to capillary congestion. The minute venules everywhere were engorged. The mucosae were dull red in color. There was blood-tinged fluid within the bowel tract and in the serous cavities.

The lungs were irregularly congested and their weight was increased. They contained innumerable small hemorrhages. Capillary hemorrhages were also present in the serous surfaces, the endocardium and in the lining of the gall-bladder and urinary bladder.

A man, aged thirty-four, took an unknown amount of "sedormide." Profound narcosis resulted and he was unconscious for eighteen hours. On admission to the hospital the red cells were 6,030,000 and the hemoglobin 118 per cent. On the following day the temperature was 97°, the pulse 130 and the blood pressure 110/70. There were moist râles throughout the chest and the percussion note was hyperresonant. Adrenalin, atropine, caffeine and pituitrin were given as stimulants. Fluids were given by mouth, by hypodermoclysis and by glucose solution intravenously. These were ineffective. Death occurred within seventy-two hours. The postmortem findings were characteristic of shock. There was general engorgement of all the body viscera. The venules and capillaries of the entire gastrointestinal tract were distended in both the peritoneal and mucosal surfaces. The lungs were extremely dark red, were almost airless and contained quantities of bloody fluid. Petechial hemorrhages were numerous throughout the lungs and in serous surfaces. Excepting the circulatory changes described, and the beginning of bronchopneumonia, microscopic examination showed no significant changes in any organs, including the central nervous system. It is stated⁹ that overdoses of sedatives belonging to this group of urea derivatives, cause a marked fall in temperature and of blood pressure due to paralysis of the peripheral vessels. Krogh's experiments¹⁰ with urethane showed that it affected the capillaries producing marked dilatation. He stated that other narcotics produce similar effects. Our microscopic examinations showed marked dilatation of the capillaries and venules in deaths from barbital and from "sedormide." Both the physiologic disturbances and the postmortem findings in such cases are indistinguishable from those of shock.

We have seen identical changes in dogs following the intravenous injection of killed bacterial cultures and following peritonitis experimentally produced. We were able to produce the typical shock syndrome in dogs by the intravenous or intraperitoneal injection of watery extract of normal dog muscle, by introducing muscle substance into the peritoneal cavity or by mechanical trauma to the limbs. In every instance the syndrome was accompanied by increased concentration of blood (Table I). In each instance the visible circulatory changes characteristic of shock were present. These findings support the conclusion that a wide variety of agents are capable of causing capillary damage, and that such damage results in circulatory failure with manifestations resembling traumatic shock.

Circulatory failure of this type, often spoken of as collapse, occurs in various clinical conditions. Toxemia with shock following intestinal obstruction is a common occurrence. It has been attributed to the absorption of histamine from the injured bowel. Underhill¹¹ noted circulatory collapse following gas poisoning and in influenzal attacks of extreme severity. In each of these instances increased concentration of blood was recorded. Atchley¹² noted the

shock syndrome in diabetic acidosis, in severe infections, and in one instance of a rattlesnake bite in which the venom apparently was injected directly into a vein.

TABLE I

CHANGES IN BLOOD CONCENTRATION IN A DOG. SHOCK WAS PRODUCED BY INTRODUCING SUBSTANCE OF DOG MUSCLE INTO THE PERITONEAL CAVITY. DEATH RESULTED IN 10.5 HOURS
BLOOD CONCENTRATION: SHOCK

TIME	SP. GRAY.	HEMOGLOBIN	RED CELLS
6:30 A.M.	1.060	101	5,110,000
7:00 A.M.	Muscle substance into peritoneum		
10:30 A.M.	1.065	104	4,860,000
1:30 P.M.	1.065	104	5,694,000
3:20 P.M.	1.067	108	5,695,000
4:50 P.M.	1.075	120	6,400,000
5:15 P.M.	1.075	130+	7,450,000

We have had opportunity to make examination following deaths from acute hemorrhagic pancreatitis, mesenteric thrombosis, intestinal obstructions, eclampsia, perforated intestinal ulcer, burns and other conditions in which the shock syndrome was a prominent antemortem feature. In each instance the blood changes and the postmortem findings were those seen in traumatic shock in man, and in experimental shock in dogs.

An experience as pathologist in a large charity hospital during the pandemic of influenza, 1917 to 1919, resulted in observations not interpreted at the time. Many of the most severe cases, which died in from two to four days from the onset, had increased concentration of blood. Red cell counts in such cases ranged from 6,000,000 to 8,000,000 and the hemoglobin was correspondingly high. The attending physicians came to regard this as a grave sign. Those patients with high concentration died early with evidences of circulatory collapse. There was a marked decline in blood pressure, weak pulse, rapid respirations and the temperature was lower than seen in cases less severe. Postmortem examination showed wet bloody lungs and extreme capillary congestion of the mucosae of the pulmonary and gastrointestinal tracts. The patients having moderate concentration of blood lived from seven to fifteen days. On postmortem examination the congestion and edema were not so extreme but were marked. Bronchopneumonia which was very irregular in its characteristics and distribution, was a regular feature. In the most severe cases no pneumonia was demonstrable either grossly or microscopically. The lungs had all the features seen in patients dying within two or three days from severe burns or from physical trauma. They presented no essential differences from the lungs following shock produced experimentally.

A young woman suffering from acute hemorrhagic pancreatitis was brought to the hospital. She had rapid weak pulse, rapid respirations, cold pale skin and falling blood pressure. No operation was done; the treatment was entirely symptomatic; she died of circulatory failure within forty-eight hours. Only one blood count was made. The red cells were 6,400,000 and the hemoglobin 112 per cent. At postmortem the visible changes characteristic of shock were present. The lungs were deeply congested, edematous, and heavy.

Their capillaries and venules were distended with blood, and there were numerous capillary hemorrhages within the lungs, in the pleurae and in other serous and mucous surfaces. The abdominal viscera were diffusely deep red and the minute venules were abnormally prominent. DeTakats and Mackenzie¹³ reported 9 cases of hemorrhagic pancreatitis, in all of which the red cells were about five million and the hemoglobin correspondingly high. One case had



Fig. 1.—Photograph of lung in shock, death within forty-eight hours following severe burn. Normal lung (left) for comparison.

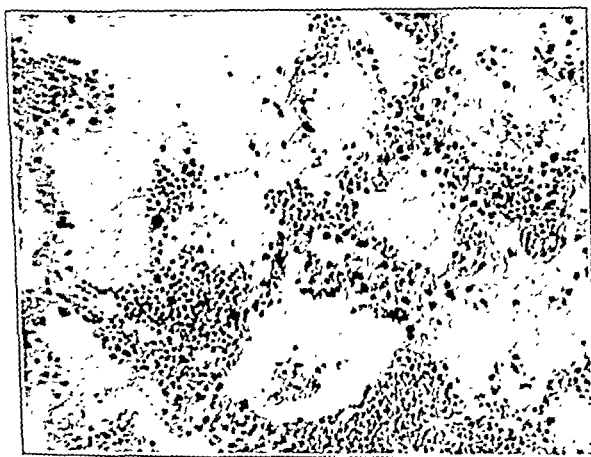


Fig. 2.—Microphotograph of lung, shock following burns. Congestion, edema and capillary hemorrhage are shown.

8,300,000 red cells and 140 per cent hemoglobin. They recognized the shock syndrome in each of these cases and stated that blood counts furnished a means for estimating the degree of shock.

Underhill¹⁴ and his associates have emphasized increased concentration of blood as a clinical feature in burns. They failed to note the relationship of this to the mechanism of shock, but recognized it as a grave physiologic disturbance and applied suitable measures for its relief. The condition is well exemplified in the following cases:

Three men were seriously burned following a collision in which a gasoline tank was burst and ignited. The burns were of the second and third degrees involving chiefly the legs, arms, and faces. Shock was a marked feature in each case. The burns varied somewhat in the amount of skin involved and likewise the degree of shock varied. The man having the most extensive burn had a red cell count of 8,350,000 and the hemoglobin was 120+. The Dare hemoglobinometer which was used had no provision for higher readings. He died within forty-eight hours. The postmortem findings were typically those of shock⁶ (Figs. 1 and 2). Another, whose burns were slightly less extensive, died of secondary pneumonia eleven days later. His condition of moderate shock, as shown by blood pressure and concentration, continued during the eleven days. The circulatory changes at postmortem were the same as described. In addition there was diffuse bronchopneumonia in an early stage. This feature is especially significant. Lungs, whose circulation is impaired and whose spaces are filled with edematous fluid, present a stage already set

TABLE II

CHANGES IN BLOOD CONCENTRATION DURING RECOVERY FROM SEVERE BURNS. THE ACCIDENT OCCURRED DEC. 12, 1932; THE FIRST BLOOD EXAMINATION WAS MADE TWO DAYS LATER
BLOOD CONCENTRATION: BURN, RECOVERY

DATE	SP. GRAV.	HEMOGLOBIN	RED CELLS
12-14	1.075	120+	6,700,000
12-15	1.070	112	5,400,000
12-16	1.063	110	5,000,000
12-17	1.070	116	5,200,000
12-18	1.068	115	5,100,000
12-19	1.066	110	4,990,000
12-20	1.062	106	4,900,000
12-21	1.055	99	4,630,000
12-22	1.048	74	3,530,000
12-24	1.045	87	3,820,000

for the final act, the development of pneumonia. The third man who was burned recovered. The blood concentration, shown in Table II, declined gradually as the patient's condition improved.

SUMMARY

Increased concentration of the blood is a regular feature developing during surgical or traumatic shock.

Circulatory failure similar to shock is of frequent occurrence in conditions accompanied by toxemia. Such conditions include burns, toxemias of metabolic origin, poisoning with certain drugs, surgical emergencies such as intestinal obstruction, pancreatitis, and perforated ulcer, and infections of unusual severity.

Increased concentration of the blood, as shown by specific gravity, red cell count and hemoglobin content, is a feature in such cases. The degree of concentration apparently is proportionate to the severity of shock.

The circulatory changes seen at postmortem in such cases are indistinguishable from those of traumatic shock. These observations indicate that the mechanism of origin is essentially the same.

Observations on concentration of blood should be of value in detecting and estimating the degree of shock in clinical studies.

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METAPLASTIC CHANGES IN THE PROSTATE GLAND*

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HISTOLOGIC changes observed in 2 cases of prostatic hypertrophy were of such a character as to lead us to study the behavior of prostatic glandular epithelium.

The first case is that of a man fifty-nine years of age (No. 8,393) who was admitted to the hospital on May 17, 1931. His chief complaint was pain in the left loin and urinary retention of twenty-four hours' duration.

He had been suffering from polyuria, nocturia, and at times dysuria, and also complained of some difficulty in starting the urinary stream and periods of retention. This condition was present for more than five years. There was also loss of weight, digestive impairment, occasional night sweats and blood-streaked sputum.

Examination revealed an elderly undernourished male who appeared acutely ill. Bronchial breathing and a few moist râles were present at right apex and below the right clavicle. A presystolic murmur was heard. The abdomen was distended and presented an ovoid swelling which reached upward to the umbilicus. There was flatness on percussion over this area. Ten-

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derness was present in the left loin. No rigidity was present in the abdomen. The right Murphy sign was positive. Rectal examination revealed a large boggy mass present in the prostatic area with most of the enlargement to the left side. A small rigid catheter was introduced into the bladder with difficulty. Catheterization recovered 52 ounces of residual urine.

A cystoscopic examination showed a marked prostatic hypertrophy especially of the left lobe. There was a generalized cystitis.

White blood cells 16,500, polymorphonuclears 78 per cent, lymphocytes 18 per cent, monocytes 4 per cent, hemoglobin 93 per cent, red blood cells 4,000,000. Urine examination on admission was negative except for a trace of albumin. Blood chemistry showed urea nitrogen 38.5, creatinine 1.5, glucose 110, chloride 460.

On May 19 a suprapubic cystostomy was performed and a mushroom catheter placed in the bladder. The patient had a rather stormy postoperative course because of the myocardial weakness and finally developed pneumonia. He died on June 15.

No consent for a general autopsy was obtained, but we succeeded in removing the bladder and prostate through the surgical incision.

Macroscopically the prostate was considerably enlarged, particularly in its transverse diameter, while the anteroposterior diameter was but slightly increased. The gland was therefore of an elongated oval shape and appeared rather flat. Its size was about twice the normal and felt quite hard to the touch. The central portion showed a superficial irregular defect, the bottom of which was covered with some grayish yellow cheesy material. On cutting through this defect it was found that the grayish yellow discoloration extended irregularly to the depth and seemed to demarcate rather sharply in the form of a saw edged line, from the lateral apparently intact parts of the prostate.

Microscopic Examination.—There was an extensive necrotic area in the center with some leucocytic infiltration of the periphery and some small irregular hemorrhages. In the lateral portion the glands were of the type seen in ordinary prostatic hyperplasia. Their epithelium was high columnar and showed a clear faintly staining cytoplasm. Many glands, however, were lined with a very low cuboidal or flat epithelium and some glands were distended thereby forming cysts, quite a few of which were of considerable size. The amount of stroma was increased throughout, and it was of a fibrous hyaline character. Rather massive fibrous tissue was found in the vicinity of the necrotic focus. The glands which persisted in this scarry area were obviously atrophic, their lumen narrow and often collapsed. Some were transformed into solid epithelial trabeculae. The cytoplasm of these cells was scanty; the nuclei were comparatively large, well stained and round or spindle shaped. There were several solid islands of squamous epithelium between these atrophic glands, some of which showed central parakeratosis while others were almost completely keratinized with the exception of a peripheral seam of cuboidal cells forming a single layer (Fig. 1). In other groups of squamous cells hydropic vacuolization could be visualized. There were also a few small

cell groups in which only the central cells looked like squamous epithelium, whereas the periphery was formed by smaller cells of the basal cell type which showed striking palisading of their nuclei. Finally there were trabeculae consisting of squamous epithelium, scattered in the fibrous tissue and alternating with groups of smaller less differentiated cells. The general appearance of these foci resembled somewhat a scirrhous carcinoma.

Summarizing our findings, we found we were dealing with a glandular hyperplasia of the prostate with interstitial fibrosis and a central focus of anemic infarction, probably due to thrombosis. The fibrotic areas revealed regressive changes of the glandular epithelium, followed by proliferation and metaplastic transformation into squamous epithelium. Parakeratosis was quite conspicuous.

The second case was that of a man aged forty-four years (No. 8,063) who was admitted to the hospital April 20, 1930, complaining of difficulty in urina-



Fig. 1.—Island of squamous epithelium with parakeratosis (high power).

tion. The patient's illness dated back about two months when he first noticed nocturia and some hesitancy in starting the urinary stream. About four weeks later pain and burning occurred when he voided.

The physical examination was negative except for some tenderness suprapubically. There was a left lumbar scar of a previous nephrectomy. A positive right Murphy sign was present. Examination of the genitalia revealed a hypospadias. On rectal examination a large tender boggy prostate was palpable.

Urine examination showed a trace of albumin and some white blood cells. Blood chemistry showed urea nitrogen 50.3, creatinine 2.9, glucose 143 mg. per 100 c.c.

Cystoscopic examination revealed a marked chronic cystitis with a constriction of the membranous urethra. On pyelogram the right kidney was somewhat enlarged and ptosed. The right ureteral catheter entered the pelvis easily. The left kidney was not present.

On April 23, 1930, a suprapubic cystostomy was performed under spinal anesthesia and a Pezzar catheter sewed into the bladder. Postoperatively the patient's condition improved. This was shown by the decreasing nitrogen retention. On May 9 urea nitrogen was 31.7, creatinine 2.5.

On May 10 a prostatectomy was performed under spinal anesthesia. The patient's condition remained satisfactory until May 13 when he began to hiccup, his abdomen became distended and the temperature began to rise. In spite of the treatment instituted he did not respond well. At this time blood chemistry showed urea nitrogen 44.3, creatinine 5.0, glucose 186. He developed uremic coma with an accompanying hypostatic congestion of the lungs and died May 22, 1930.

The prostate removed was comparatively small, flat and adherent to the surrounding fat tissue. It was very hard and showed a fibrous structure on section with quite a few scattered irregularly formed narrow fissures which occupied mainly the central portion of the more superficial stratum.

Microscopically a fibrosed prostate was found with considerable lymphocytic infiltration, particularly about the very irregularly formed glands. These glands did not resemble the ordinary prostate glands. Some were tubular, others irregularly branching. They were lined with multiple layers of squamous epithelium often causing the lumen to become quite narrow and usually eccentric. Proliferation of the epithelium sometimes closed the lumen altogether and yielded solid epithelial structures. The epithelial cells were usually small, but with quite large nuclei which were round or oval and fairly intensely stained. In other places, particularly in the solid epithelial nests, the cells were smaller, the nuclei densely packed, darker stained, and rod or spindle shaped, thereby resembling basal epithelia.

Careful examination of many slides showed that particularly in the lateral portions a few glands could be found which revealed the ordinary prostatic epithelium with the folding and formation of papillae characteristic of hyperplastic prostatic glands. In some of these glands, transformation of the cuboidal or high columnar epithelium into much smaller cells with darker nuclei could be ascertained. These cells formed several layers and gradually transformed the ordinary appearance of the prostatic gland into such tubular structures as described above.

Summarizing these findings we concluded that we were dealing with a chronic fibrosis of the prostate, apparently of inflammatory nature with transformation of practically all the prostatic glands into tubular structures, lined with squamous epithelium or solid groups of squamous or basal epithelium.

Metaplastic changes of glandular epithelium concomitant with hyperplasia are known to occur in almost any glandular organ. This is the most logical explanation of the observation that glands or mucous membranes covered with columnar epithelium reveal foci of basal or squamous cells. This process was first described in the lungs by Goldzieher who showed that in chronic inflammatory conditions the columnar bronchial epithelium reverts first to cells of the basal type, the proliferation of these cells yielding occasionally considerable thickening of the mucosa and eventually occlusion of the bronchial lumen. The squamous cells form secondarily through differentiation

of the basal cells. This interpretation is based on the conception that the basal cell represents an indifferent type of epithelium to which the columnar cells may revert under unfavorable conditions. The basal cell is considered to be a multipotent cell, which is capable of differentiating into any other type of epithelium.

Goldzieher's explanation of bronchial metaplasia and hyperplasia of the



Fig. 2.—Squamous epithelium all along the wall of the prostate glands. In the center a solid island of squamous epithelium (low power).



Fig. 3.—Incipient basal cell proliferation substituting the columnar cells of the prostatic gland (high power).

basal cells has been accepted by all the later workers (Teutschlaender, Askanazy, Smith, Fried and others). The experimental studies of Wolbach and Howe have shown, furthermore, that changes similar to those seen in chronic infections can be produced in animals by a diet deficient in vitamins. Basal cell hyperplasia with concomitant metaplasia has been described since in other organs, such as, the pancreas and gallbladder (Krompecher, Roesiger).

The occurrence of solid epithelial nests and of squamous epithelium in the prostate is known since Aschoff demonstrated the physiologic growth of squamous epithelium during the late stage of fetal life. His work was corroborated by Schlachta, who is also of the opinion that remnants of this fetal squamous epithelium may persist in the adult. Schmidt was the first to observe extensive parakeratosis in such islands of squamous epithelium found



Fig. 4.—Advanced basal cell proliferation with beginning differentiation into squamous epithelium (high power).



Fig. 5.—Massive proliferation of basal cell, practically obliterating the glandular lumen (low power).

in the adult. He interpreted this lesion in accord with the conception of Schlachta, although these changes were found in close vicinity to an old prostatic abscess. Similar views were held by Tietze and Garfunkel, who observed squamous epithelium in the prostate of eunuchoid and also believed he was dealing with a malformation. Krompecher, however, in a detailed study on the prostatic glandular epithelium showed that there is no need for embryonal nests nor for the hypothetical malformations to explain the histo-

genesis of such squamous epithelium. Their origin from the ordinary glandular cells, or from the epithelium of the prostatic ducts can be clearly demonstrated. The process involved is identical with that which occurs in the bronchial mucosa. According to Krompecher the occurrence of such basal cell hyperplasia and secondary metaplastic differentiation into squamous epithelium is quite common and seems to evolve from regenerative processes in the course of chronic inflammation. In a large series examined for the presence of such epithelial changes, they were observed in 45 per cent of the cases. These figures coincide with our own observation. In reviewing 26 prostates removed during the last eighteen months, 11 cases (that is 41 per cent) show more or less marked hyperplasia with metaplastic changes. Other authors (Grassmann, Neller and Neuburger) give lower figures and estimate the frequency of what they term "atypical epithelial proliferation" at 25 per cent.

There is some difference of opinion as to the actual seat of the lesion. Wagenseil and Altmann believe that the squamous epithelium develops from the ducts of the prostate, whereas, Krompecher found the changes as a rule in the glandular epithelium proper. Our own observations bear out Krompecher's statements, as we frequently observed glands lined partly with ordinary epithelium, while other parts of the same gland already showed definite basal cell hyperplasia. The sections, described in this paper from which the photographs (Figs. 1 to 5) were made, were taken from the lateral portions of the gland so far away from the urethra that there could be no suspicion that the tissue described was urethral glandular tissue. The occurrence of parakeratosis in our first case is the third observation of this kind on record, including those of Schmidt and Altmann.

The changes described in this paper are not only morphologically interesting, but they are also of clinical importance. The presence of squamous epithelium, with or without keratinization and the formation of solid nests and plexi of basal cells in a place where such epithelium does not occur physiologically, may erroneously lead to the assumption of the presence of a neoplastic process. It has been stressed recently by Grassmann that these atypical epithelial formations closely resemble carcinoma, and it is often very difficult to make the differential diagnosis between such lesions and early cancer of the prostate. As a matter of fact there is a frequent coincidence between such changes and carcinoma. This has been noticed by Krompecher, who held the opinion that carcinoma of the prostate, if not of the adenomatous type, is preceded by and develops from such basal cell vegetations. This would explain the occurrence of both basal and squamous cell carcinoma of the prostate. Krompecher's interpretation is based not only on his direct observations in carcinoma of the prostate but also on the analogy with similar observations in other organs, particularly the lungs. A recent paper by Fried also emphasizes the importance of bronchial basal cell hyperplasia for the histogenesis of carcinoma of the lungs. This had already been suggested by Goldzieher.

SUMMARY

Two cases of extensive metaplasia of the prostatic glandular epithelium are described. The histogenesis of metaplasia is discussed in its relationship

to basal cell hyperplasia. The frequency of these changes in the prostate and their significance in the development of prostatic carcinoma is emphasized.

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LABORATORY METHODS

THE DIAGNOSIS OF SURGICAL TUBERCULOSIS*

RELATIVE RELIABILITY OF LABORATORY METHODS

ROBERT McCULLEY HALBACH, Sc.M., M.D., TOMS RIVER, N. J.

AT THE New York Orthopaedic Dispensary and Hospital a considerable number of patients are seen with symptoms in a bone, joint, or associated soft tissue which indicate a probable tuberculous lesion. As the pioneer surgery of Dr. Russell A. Hibbs has thoroughly proved, the treatment *par excellence* in such cases is fusion of the affected joint. This presupposes that the diagnosis has been confirmed by methods that are subject to the least possible error.

The ideal method should demonstrate tuberculous infection whenever present and be equally reliable in its negative phase, but as yet no single test has reached this degree of perfection. However, by the judicious combination of several methods, each of considerable accuracy in itself, we believe errors in diagnosis can be reduced to a rarity, indeed, practically excluded.

In demonstration of this contention, we are reporting the results of laboratory examinations made upon all material received from April, 1930, to October, 1932, in which tuberculosis was the object of search. Results are divided into Period A (from April, 1930, to September, 1931, inclusive) and Period B (from October, 1931, to October, 1932, inclusive) since the technic was changed in the latter group. All specimens were examined or confirmed by the author.

TABLE I
PERIOD A—TISSUES (TOTAL 103)

Histology positive—Guinea pig positive	52
Histology positive—Guinea pig negative	7
Histology doubtful—Guinea pig positive	1
Histology doubtful—Guinea pig negative	0
Histology negative—Guinea pig negative	37
Histology negative—Guinea pig positive	1
Histology not supplemented; intercurrent death of pig	5

That is, inoculation confirmed 89 diagnoses, changed 2 to positive and failed to confirm or supplement 12, a percentage reliability of 88.4. Of the

*From the Department of Pathology; New York Orthopaedic Dispensary and Hospital. Received for publication, March 1, 1933.

7 animals which were negative while the corresponding tissue was positive by section, it is most probable that the tissue for inoculation was insufficiently macerated or injected in inadequate amounts, since in three cases subsequent tissue from the same lesion area yielded positive animals. In the tissue which was negative histologically but from which a positive pig was obtained, the material for section came from the head of the femur and that for inoculation from the ilium.

TABLE II
PERIOD A—JOINT FLUID OR PUS (TOTAL 69)

Guinea pig positive	45
Guinea pig negative	19
Guinea pig not supplementary—intercurrent death	5

Percentage reliability in this group was 92.8. Of these 69 specimens, positive animals were obtained in all that revealed acid-fast bacilli by staining. There were 41 which also had tissue sectioned from the same lesion area and the diagnoses agreed in 35, 3 were positive while the fluid or pus was negative, and 3 could not be checked because the pig with corresponding pus died of intercurrent disease.

In summary for Period A, a total of 172 specimens were examined. In 153, animal inoculation confirmed other methods, in 2 it changed a diagnosis to positive, and in 17 it failed to supplement, a percentage reliability for this period of 88.9. Moreover, in at least 3 cases examination of tissue from exploration revealed tuberculosis when the aspirated fluid or pus had been negative.

The factors of intercurrent deaths of some pigs and failure of others to confirm definite histologic evidence of tuberculosis in the above series constituted a serious defect, and in October, 1931, we adopted the technic given at the close of this report, and with the following results:

TABLE III
PERIOD B—TISSUES (TOTAL 57)

Histology positive—Guinea pig positive	33
Histology positive—Guinea pig negative	0
Histology negative—Guinea pig negative	20
Histology doubtful—Guinea pig positive	2
Histology doubtful—Guinea pig negative	1
Histology supplemented—pig died of pyogenic infection	1

That is, inoculation confirmed 54 diagnoses and settled 3 otherwise doubtful diagnoses. Percentage reliability of inoculation in this group was 100. In each of the 2 tissues doubtful by section both animals died of tuberculosis and in the one tissue doubtful by section both animals were negative. In the tissue classed as supplemented, both animals died (four and six days after inoculation) of a staphylococcus peritonitis, the tissue by section was a definite pyogenic inflammation and culture of tissue yielded staphylococci. In 4 other specimens one animal died of intercurrent disease but its duplicate subsequently died of tuberculosis or was negative by autopsy after sixty days.

TABLE IV
PERIOD B—JOINT FLUID OR PUS (TOTAL 28)

Guinea pig positive	18
Guinea pig negative	10
Guinea pig nonconclusive	0

Percentage reliability of inoculation in this group was 100. All material in which acid-fast bacilli were found yielded positive pigs. In 17 cases tissue from the same lesion area was examined and the diagnosis agreed with the findings from fluid. Also in this group 4 animals died of intercurrent disease but the duplicate remained to complete the diagnosis.

During the interval covered by both periods there were also 8 spinal fluids inoculated into animals with no intercurrent deaths; and 18 urine sediments inoculated with one death from intercurrent disease in period A. This last animal, however, did not develop pneumonia until the fifty-fourth day after inoculation and at autopsy its spleen, liver and lymph nodes were negative, so that it may properly be considered as negative for tuberculosis.

From the results in Period B we therefore feel that tuberculosis of the bones, joints and associated soft tissues can be diagnosed or excluded with great reliability if the following methods of examining material are employed:

Tissue.—In the absence of conclusive evidence from pus, fluid, etc., and with the physical findings indicative of tuberculosis, exploration is performed and several small portions of suspected tissue removed. A frozen section of a portion is made at once and, if diagnosed positive by an experienced observer, any therapeutic surgery that may be indicated can be proceeded with. In such cases completion of tissue section by paraffin or celloidin is the only confirmation needed. Without frozen section or when it is not clearly positive, a portion of tissue is expeditiously sectioned by celloidin or paraffin and, pending its completion, a similar portion prepared as follows:

Place tissue in sterile glass mortar, add from 5 to 8 c.c. of sterile saline and a small amount of sterile emery (medium) powder, and macerate thoroughly; add an equal amount of 6 per cent sulphuric acid, mix and place in 37° incubator for thirty minutes; transfer all but coarse fibers and emery sediment to centrifuge tube with a pipette and run at high speed (from 2,500 to 3,000 r.p.m.) for thirty minutes; remove supernatant fluid, resuspend sediment in 6 c.c. of sterile saline and set aside in ice box.

Sections should be completed within three to four days and, if cytology is clearly tuberculous, no further confirmation is needed and prepared sediment in ice box is discarded. When sections are negative or doubtful, proceed with animal inoculation of prepared sediment as follows:

Use guinea pigs of from 250 to 300 gm. that have been quarantined for three weeks following receipt in laboratory. For each sediment suspension inoculate two pigs both intraperitoneally, giving one 2 cc. and the other 4 c.c. Each animal is housed in a separate cage and observed daily. Inoculated pig is killed when appreciably ill or at the end of sixty days. When autopsied by an experienced observer, typical gross tuberculous lesions of spleen, liver, etc., are considered conclusive; otherwise, or when gross lesions are doubtful, acid-fast stains on smears made from involved viscera shall be the final criterion.

Pus.—Routine cultures and smears for Gram's and acid-fast staining are made. The remainder, up to 5 c.c., is treated with an equal amount of 6 per cent sulphuric acid, etc., as under preparation of tissue sediment, and the resuspended sediment inoculated into two animals in like amounts.

Joint Fluid.—Immediately on withdrawal mix with several cubic centimeters of sterile sodium citrate ($2\frac{1}{2}$ per cent) solution to prevent coagulation and then proceed as with pus.

Spinal Fluid.—Unless distinctly turbid or pyogenic organisms found in smears, inoculate directly into two animals, using 3 c.c. and 6 c.c. respectively. If turbid, or pyogenic organisms are present, treat a 10 c.c. portion with sulphuric acid, etc., as under pus, resuspend sediment in 6 c.c. of saline and inoculate in amounts of 2 c.c. and 4 c.c.

SUMMARY

Laboratory methods for the diagnosis of surgical tuberculosis and results of their use are given which, the author believes, warrant the following conclusions:

1. By these methods tuberculosis can, with rare exceptions, be definitely diagnosed or excluded.

2. Negative results by any or all of these methods in the case of joint fluids or pus do not completely exclude tuberculosis. In such cases, where the physical findings, etc., remain suspicious, exploration and examination of tissue are indicated.

513 MAIN STREET.

IMPROVED DRINKING FOUNTAIN FOR SMALL LABORATORY ANIMALS*

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IT IS convenient to attach the water reservoirs to the outside of the doors when cages are stacked closely, and one upon the other. The inverted bottle type of drinking fountain is inexpensive and lends itself readily to this purpose.

Large bottles require simple curved or angulated glass nipples. Bottles of not more than 100 c.c. capacity are required for cages with doors only 8 inches high, in order that they will not prevent the opening of the door of the cage immediately above. The partial vacuum created by the inversion of the small bottle using simple curved or angulated nipples may impede greatly, or prevent entirely, the flow of water. The nipple shown in Fig. 1 was devised and has proved satisfactory in overcoming this difficulty.

It is made of glass tubing 9 mm. in outside diameter, and is blown in the form of a gentle curve instead of with an angulation. The lower part is

*From the Gynecean Hospital Institute of Gynecologic Research, School of Medicine, University of Pennsylvania.

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tapered to approximately one-half of its original diameter, and the open end is pressed into the shape of an hourglass.

The hole at the middle of the nipple is its most important feature. As the animal drinks by licking the end of the nipple, bubbles of air form at the hole in the midpoint of the nipple, enter, glide easily around the curve and rise to the surface of the water where they tend to equalize the partial vacuum. As a result, the animal has no difficulty in drinking.

The curve of the nipple facilitates the uninterrupted movement of the bubbles of air.

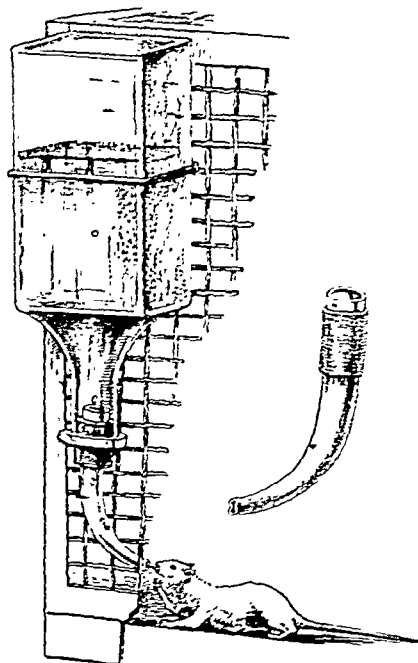


Fig. 1.—Showing construction and mode of operation of glass nipple suitable for 100 c.c. inverted bottle type of drinking fountain, attached to the outside of cage door. Note especially the 1 mm. diameter hole in the nipple at the midpoint of the curve and upon its concave aspect; also that the bubbles of air form at this point as the animal drinks.

The position and size of the hole, however, are the most important considerations in the construction of the nipple. The best site for the hole is on the concave aspect of the curve at its midpoint. Its optimum diameter is 1 mm. If the hole is smaller, the bubbles form with great difficulty if at all. If the hole is larger than 1 mm., they form more readily, are larger and more numerous, but when the animal stops drinking, they continue to form and the bottle empties automatically.

The credit for developing the nipple belongs to Charles W. Miller, glass blower, The Laboratories, Philadelphia General Hospital.

A SIMPLE AND EFFICIENT REFLUX CONDENSER*

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IN THE determination of cholesterol in blood serum and plasma, reported in a previous communication,¹ it was found that the test tube condenser utilized was subject to certain mechanical difficulties. The water passing through the condenser had to be carefully regulated so that the pressure would not be too great; also, a slow boiling water-bath was essential to prevent condensation of steam into the Folin tube with subsequent interference with the color formation.

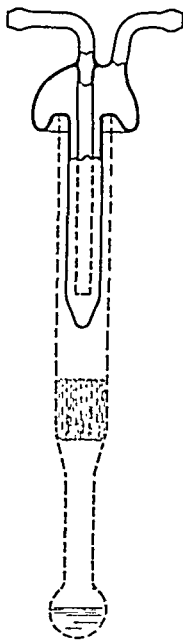


Fig. 1.

All defects were obviated by the use of the condenser shown in Fig. 1. The entire condenser is a single unit of glass, the top of which is made mushroom shape.

The condenser can be used with a vigorously boiling steam bath, and with a greater rate of flow of the condenser water, thereby increasing the efficiency of the condenser.

The particular shape of the condenser and the manner in which it fits the top of the Folin tube prevent any condensation of steam on the inside of the Folin tube.

The condenser was made by Eimer and Amend, New York, N. Y.

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CYTOLOGIC STUDY OF BODY FLUIDS*

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AN INFREQUENTLY practiced but highly valuable aid to diagnosis has reappeared in the literature following a lapse of several years. It is not at all uncommon for the medical practitioner to encounter a patient with a seemingly unaccountable effusion in one of the body cavities. That mechanical and metabolic factors, nonneoplastic in nature, may be the cause of such an effusion is a well-established fact. It is also known that certain inflammations, bacterial, chemical, or otherwise, may produce this fluid. It becomes the problem of the practitioner, in such an instance, to determine whether the effusion is due to one of the above factors, or whether it is produced through the mechanism of a neoplasm of the serous membrane or its circulatory supply. Roentgenologic study in these cases is usually of little value unless the fluid is removed before radiography. Even then, the uncertain data gathered from the x-ray (as to the nature of the dyscrasia, whether inflammatory or neoplastic, and if neoplastic, whether primary or secondary) can be transformed into certain knowledge by a study of the cells found in that fluid. For these occasions, it has become possible, in recent years, to offer a valuable and quite accurate aid to the diagnosis, in the rôle of a cytologic study of the fluid sediment.

Since the publication of Mandlebaum's technic for cytodagnosis of serous fluids appeared in 1917, it has been possible, with a considerable degree of accuracy, to rule out cardiac, inflammatory and metabolic disturbances, to determine whether a given serous effusion has a neoplasm as its source.

Mandlebaum's achievement followed upon the investigations of many workers who slowly, and step by step, prepared the field for the modern-day methods of single tissue cell diagnosis. Attempts to identify tumor cells in body fluids date back to 1860 when Beale² first noticed large multinucleated cells in unstained smears of sputum in a case of carcinoma of the pharynx. Little attention was paid to this, as well as to the work of Quinke³ and of Ehrlich,³ each of whom in 1882 demonstrated with new staining methods the presence of tumor cells in smears of pleural and peritoneal effusions. The first attempts at using cut sections were made in 1895 by Bahrenberg¹ who allowed a large quantity of fluid to clot spontaneously and then hardened the clot in alcohol, imbedded it in celloidin and cut it as a tissue. In 1900, Mandlebaum⁴ demonstrated the presence of actinomyces in pus, by means of tissue-like sections, and it is this method which he described in 1917⁵ as being of value when seeking tumor cells in fluids, and which has become the foundation upon which all later methods were based.

*From the Pathological Laboratories, Sydenham Hospital.
Received for publication, April 19, 1932.

Mandlebaum's method, as practiced at Mount Sinai Hospital in New York was the basis for a very exhaustive report in 1928 by Zemansky,⁷ who evaluated 914 examinations made at that institution over a period of fifteen years. His conclusions show that this study can be of inestimable value in arriving at a diagnosis. He emphasized also the worth of a positive report, stating that a negative report was not as conclusive, since the cells could be destroyed or missed even when present. His positive results, however, he regarded as being almost pathognomonic, since in the presence of a sheet of epithelial cells or of only a single cell with mitotic figures, an accepted diagnosis of neoplasm could be made. His checked results bore out these conclusions. At this point it may be advisable from our experience to add a cautioning statement: occasionally a sheet of endothelial cells may be present, having desquamated from the serous membrane. This may closely resemble squamous epithelium or even the medullary cells in a solid carcinoma (Fig. 1).



Fig 1.—Note sheet of large, pale, peritoneal cells. These are not neoplastic.

In his large series of cases Zemansky was able to arrive at an accuracy of 90 per cent in his positive results as compared to an accuracy of only 47 per cent in his negative results.

In the much shorter series that we have to report, we have been more fortunate in gaining a somewhat higher percentage of correct results. Over a period of five years, we have had 26 patients on whom we have made 39 examinations. Of these there were 32 positive diagnoses, with one case disproved (Case 25), for an accuracy percentage of 96.9 per cent. In only 7 cases were negative diagnoses made, and these were all checked as nonneoplastic, for an accuracy percentage of 100 per cent. From these statistics it can be seen that in all our cases where tumor cells could have been expected to be present they were found at the microscope. In no positive case was the diagnosis missed, as all our negative results were checked as such by the subsequent history of the case.

Though our series is too short to make the accuracy percentages definitely acceptable, we feel that our results have been so good as to justify the describing of our method, which is a modification of Mandlebaum's, and which we be-

lieve more readily preserves the cells, thus increasing the number of positive diagnoses, decreasing the number of negative diagnoses, and giving a greater general accuracy in cytologic study by this method.

Technic.—The original technic as described by Mandlebaum consisted in preparing the fluid sediment for cutting as a tissue section. This he did by centrifuging the fluid in large conical tubes and getting a sediment which he hardened in 10 per cent formalin and then ran through the alcohols, through chloroform and into paraffin. The hardened block of sediment was then cut, stained and mounted as a tissue section.

The accuracy of the positive results is well attested to by Zemansky's experiences, but the negative results are painfully inaccurate. The reason for this is obvious. In any test where the result depends on the finding of a few isolated cells, a number of factors may enter to cloud the picture. It is possible, for instance, to have a neoplasm involving the pleura with a consequent effusion, and yet have no tumor cells in that fluid. It is possible that no cells have desquamated. It is also possible that so few cells have desquamated that they are extremely difficult to find. For that reason Mandlebaum cautioned that as much fluid as is possible should be obtained and centrifuged. Another possibility is that the tumor cells, subjected to a prolonged sojourn in an environment to which they are certainly not adapted, may be changed to a considerable extent, and that the strenuous regime of fixation, dehydration, clearing, and infiltration to which they are subjected, may so further distort them as to make them unrecognizable, or that these processes may destroy the cells completely. It is true that most frequently the cells remain recognizable in sufficient numbers to permit a correct diagnosis. But, as Zemansky stated, it often happens that one gains an impression that there is a malignancy present, while still unable to give a diagnosis, and also one occasionally gives a negative diagnosis, only to find on subsequent examination or in the future course of the case that a neoplasm was present.

It is for these reasons that we have slightly modified Mandlebaum's technic, seeking to produce less strain on the chemical and physical properties of the neoplastic cells, and thus more often preserving them for diagnosis. The method employed is as follows:

1. Obtain sediment by centrifuging the fluid in large conical tubes at from 1,200 to 1,700 revolutions per minute for from one-half to one hour. Wash sediment several times in normal saline solution.
2. Decant supernatant fluid. Replace with about 25 times the volume of 5 per cent formal acetic acid, at 37° C. for from four to six hours.
3. Dehydrate by steeping through the following solutions:
 - a. 75 per cent alcohol for one hour
 - b. 95 per cent alcohol for one hour
 - c. 2 parts absolute alcohol and 3 parts ether for one-half hour
 - d. ether for one-half hour
 - e. 2 parts ether and 3 parts acetone for one-half hour
 - f. acetone for one-half hour

4. Clear in choloroform.
5. Infiltrate with paraffin at 45° C. gradually increasing the temperature so that in two hours it reaches 56° C.
6. Embed in paraffin as with ordinary tissue blocks.
7. Cut with sharp knife at slight angle, thus exposing a greater surface of the sediment for staining.
8. Staining and mounting is carried out as for routine tissue work.
9. Cut many slides to decrease chance of missing cells.

Comment.—1. Five per cent formol acetic acid is used as a fixative in place of 10 per cent formalin because the latter fixes the cells too rapidly and may produce considerable distortion, while the former permeates through the cells more slowly without injuring their structure.

2. The sediment is dehydrated more slowly and in a graded manner, again avoiding rapid, distorting changes in their appearance.

3. Cutting the block with the knife edge at a slight angle exposes a greater surface of cells and nuclei for staining.

CASE REPORTS

CASE 1.—Mrs. R. D., a white female aged forty-eight years, was admitted to Sydenham Hospital on Oct. 7, 1932, with symptoms of an enlarging abdomen for some weeks and

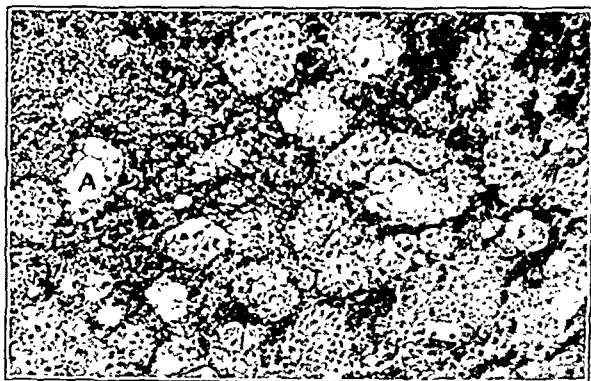


Fig. 2.—Authors' Case 1. Abdominal fluid. Diffuse peritoneal carcinomatosis. A. Note acinus formation.

with urinary disturbances. A roentgenologic series was of no aid. There were 8,000 c.c. of straw-colored fluid in the abdomen, which on examination by the above method showed adenocarcinoma (Fig. 2). Laparotomy revealed large thick, white patches on the parietal peritoneum and between the uterus and urinary bladder. The cecum was immovable. Microscopically these patches showed diffuse carcinomatosis.

CASE 2.—Mrs. J. G., a white female aged sixty-nine years, was admitted to the Sydenham Hospital on Aug. 23, 1932, with a pleural effusion on the right side. A chest tap was productive of 1,600 c.c. of light amber fluid, which showed many small clumps of dark staining epithelial cells. A diagnosis of metastatic carcinoma was made, and again at two subsequent tappings, at which occasions bloody fluid was removed. During this time it was learned that four years previously the patient had had a left-sided mastectomy for carcinoma.

CASE 3.—Mrs. V. M., a white female aged twenty-nine years, was admitted to the Sydenham Hospital on June 2, 1932, with the diagnosis of serous pleurisy, possibly tuberculous. Examination of the fluid showed the absence of tumor cells, and the presence of an exudate with a large preponderance of lymphocytes and some hyaline material resembling

caseation. No giant cells were seen. A diagnosis of probable tuberculosis was made, and confirmed by the result of guinea pig inoculation.

CASE 4.—Mrs. B. B., a white female aged forty-three years, was admitted to the Sydenham Hospital on Nov. 16, 1931, with fluid in the right chest, cough and pain on that side. A tap was productive of 750 c.c. of sanguinous fluid which showed on cytologic study large groups of neoplastic cells of the kind frequently seen in tumors of the ovary. Six subse-



Fig. 3.—Authors' Case 4. Chest fluid. A, Note mitoses; B, double nuclei.

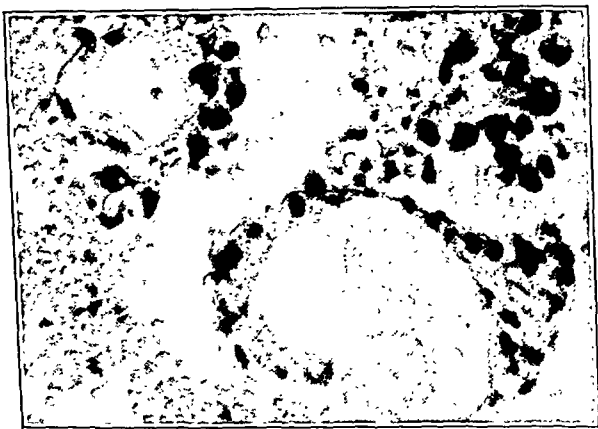


Fig. 4.—Same case as Fig. 3. Chest fluid. Note close resemblance to cells in ovarian carcinoma.

quent thoracenteses resulted in the same findings and gynecologic examination disclosed a large mass in the left adnexal region. Operation was performed and both ovaries removed. Microscopically there was a bilateral papillary adenocarcinoma of the ovaries Figs. 3, 4, and 5.

CASE 5.—Mrs. R. N., a white female aged thirty-four years, was admitted to the Sydenham Hospital on Feb. 3, 1932, with a cough and a left-sided pleural effusion. Aspiration resulted in a sanguinous fluid that showed a denocarcinoma on microscopic study (Fig. 6). The roentgenologic diagnosis after the tap was metastatic involvement of the lungs secondary to pleural malignancy.

CASE 6.—Mrs. B. F., a white female aged fifty-six years, was admitted to the Sydenham Hospital on Feb. 15, 1932, with pain in the chest, dyspnea even at rest, and paroxysms of coughing. There was no hemoptysis, and no signs of congestive circulatory failure.

Thoracentesis was productive of a hemorrhagic fluid which showed a probable pleural endothelioma. This was confirmed by x-ray, and later by necropsy at another institution (Fig. 7).

CASE 7.—Mrs. H. B., a white female aged eighty-three years, was admitted to the Sydenham Hospital on Dec. 21, 1931, with a cystic mass the size of an orange in the right kidney region. It was found to be a cyst in the sacroiliac joint. The aspirated fluid showed an epithelial malignancy. Other metastatic foci were found at radiographic study, but the primary tumor was not located.



Fig. 5.—Papillary adenocarcinoma (bilateral). Same case as Fig. 3. Ovarian tumor. A, Note resemblance to cells in chest fluid.



Fig. 6.—Chest fluid in case of breast carcinoma. A, Note ductal formation and resemblance to breast tumors.

CASE 8.—Mrs. P. K., a white female aged thirty-eight years, was admitted to the Sydenham Hospital on March 20, 1930, for parturition, with cough and pain in upper right chest. Two years previously she had had a right mastectomy for carcinoma. Roentgenologic study showed fluid in the right pleural cavity. At thoracentesis a hemorrhagic fluid was found, which on section of the sediment with microscopic study thereof showed sheets of neoplastic epithelial cells. The patient later died at another institution.

CASE 9.—Mr. M. L., a white male aged forty-two years, was admitted to the Sydenham Hospital on April 25, 1932, with generalized icterus, loss of weight, weakness, and a vesicular

rash on his lower extremities. The axillary and inguinal lymph nodes were considerably enlarged. There was a marked pleural effusion which was tapped on two occasions, and each time showed the presence of many eosinophiles, reticulum cells, endothelial cells, and a suggestion of Dorothy-Reed cells. The diagnosis of Hodgkin's disease was made, and confirmed by the subsequent course of the disease, although no biopsy was made. The patient is under a deep x-ray therapy and follows a typical Hodgkin's disease course.

CASE 10.—Mr. J. S., a white male aged forty-three years, was admitted to the Sydenham Hospital with a history of sudden ascites that came on a few weeks previously. At

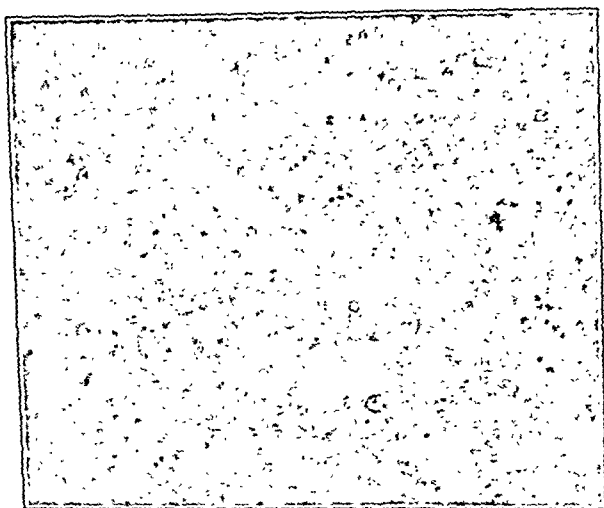


Fig. 7.—Authors' Case 6. Chest fluid. Note typical foamy cells with vesicular nuclei. A, Mitoses; B, double nuclei.



Fig. 8.—Authors' Case 8. Abdominal fluid. Note diffuse carcinomatosis. Scirrhous type arrangement.

laparotomy there were 10 quarts of fluid and a diffuse peritoneal carcinomatosis. Examination of the fluid sediment showed numerous large spheroidal epithelial cells with acidophilic cytoplasm and pyknotic nuclei (Fig. 8). The primary focus was not located.

CASE 11.—Miss M. H., a white female aged thirty years, was admitted to the Sydenham Hospital on May 3, 1929, with vague abdominal pains and ascites, associated with loss of weight and strength. The abdominal fluid was aspirated and showed numerous epithelial cells of various sizes and shapes with numerous mitotic figures. There were many large

cells, hydropic with the nuclei displaced to the periphery giving the typical "signet ring" cells (Fig. 9). The diagnosis was confirmed at operation.

CASE 12.—Mrs. B. H., a white female aged sixty years, was admitted to the Sydenham Hospital on July 3, 1929, with a history of diabetes, complaining of dyspnea, and having fluid in the chest. Thoracentesis resulted in a hemorrhagic fluid which showed microscopically many epithelial cells piled up on a fibrinous basement membrane in papillary arrangement. A diagnosis of papillary carcinoma was made, but was not substantiated since the patient left after the fluid recurred, and has not been heard from.

CASE 13.—Dr. S. H., a white female aged forty-seven years, was admitted to the Sydenham Hospital on Nov. 6, 1930, with dyspnea, cough, abdominal distention, and vague pains in the abdomen and chest. Radiograph disclosed the presence of large abdominal glands, and fluid in the chest. Hemorrhagic fluid was removed which showed many pus cells and numerous reticulum cells and eosinophiles with endothelial cells of the type seen in Hodgkin's disease. The diagnosis was confirmed by biopsy.

CASE 14.—Mr. J. S., a white male aged thirty-six years, was admitted to the Sydenham Hospital on Jan. 27, 1932, with cough, expectoration of tenacious sputum (often blood-tinged)

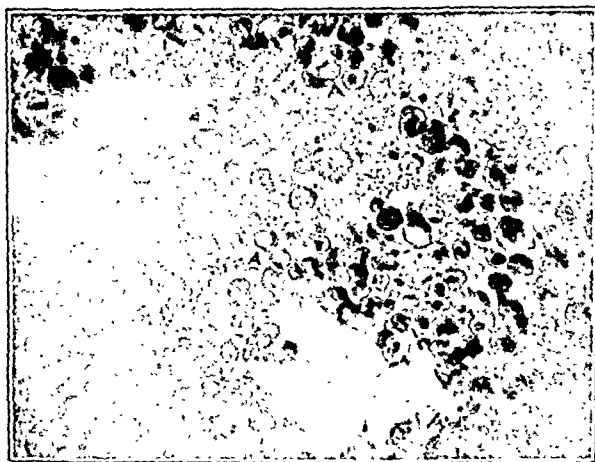


Fig. 9.—Authors' Case 11. Abdominal fluid. A. Note typical "signet ring" cells as seen in Krukenberg tumor of ovary.

and dyspnea with pleuritic pain on both sides of the chest. At thoracentesis a hemorrhagic fluid was aspirated which showed many sheets of epithelial cells, many with mitotic figures. A diagnosis of epithelial malignancy was made, and in March, two months later, bone metastases were found. The patient died shortly afterward, and since no autopsy was performed the primary focus was never ascertained.

CASE 15.—Mrs. C. C., a white female aged thirty-seven years, was admitted to the Sydenham Hospital on April 7, 1932, with a postpneumonic lung abscess, and fluid in the right chest. Aspiration was productive of a thick yellow fluid which showed no tumor cells, and many leucocytes. A negative diagnosis was made, and the patient subsequently recovered.

CASE 16.—Mrs. B. H., a white female aged fifty-eight years, was admitted to the Sydenham Hospital on Dec. 5, 1932, with precordial distress, weakness and loss of weight (50 pounds in two years). Her sputum was mucoid and blood-streaked. Large nodes were felt in the region of the bifurcation of the carotid (right). Aspiration of the fluid in the right pleural cavity produced brownish yellow fluid which showed on microscopic examination numerous spheroidal epithelial cells with round vesicular nuclei and many mitotic figures. A diagnosis of epithelial malignancy was made, and confirmed by x-ray, but the primary focus was never found.

CASE 17.—Dr. A. W., a white male aged fifty-two years with loss of weight and painless jaundice for six months. He had had a cholecystectomy two years previously, and prior to that had had a finger amputated because of an epithelioma which had developed due to his work with x-rays. The growth never recurred. Shortly before his death he developed an ascites, which was aspirated and showed a clear amber fluid with many spheroidal epithelial cells with irregular nuclei and mitotic figures. A clinical diagnosis of carcinoma of the pancreas was made, but no further substantiating evidence was forthcoming.

CASE 18.—Mrs. T. S., a white female aged eighty-five years, with urinary disturbances, frequency, dysuria. Her bladder was washed and the washings on two separate occasions examined as for cells in an effusion. The microscopic study showed many inflammatory cells and many sheets of papillomatous formations, that showed malignant changes in the cells. A diagnosis of papilloma with malignant degeneration was made and confirmed by the cystoscopic examination.

CASE 19.—Mr. A. L., a white male aged fifty-three years, was admitted to the Sydenham Hospital on April 4, 1931, with complaint of pain in the right chest. Several tapplings were productive of straw-colored fluid which failed to show any neoplastic cells, but did contain many lymphocytes and monocytes. Radiography disclosed a large mass in the middle lobe of the right lung, and a diagnosis of pulmonary neoplasm was made notwithstanding the negative reports from the laboratory and the presence of a positive Wassermann test. At autopsy a large gumma of the lung was found.

CASE 20.—Mr. S., a white middle-aged man was found by his physician to have fluid in the pleural cavity. The physician aspirated this fluid, which was hemorrhagic in nature, and brought it to the laboratory for cytologic study. Paraffin sections of the sediment showed many plasma cells and many large, foamy cells, probably endothelial in origin with double nuclei and mitotic figures. A diagnosis of probable pleural endothelioma was made, but was not confirmed as the patient was lost track of.

CASE 21.—Baby T., a white male aged three and one-half years, was brought to his physician in a state of suffocation. The doctor suspected an enlarged thymus, had the child studied radiographically, and found fluid in the pleural cavities with diffuse nodular growths in the lungs. Study of the fluid sediment showed many lymphocytes and monocytes with many reticulum cells. A diagnosis of mediastinal lymphosarcoma was made and confirmed by the x-rays and subsequent course of the disease. The child was improving remarkably with deep therapy, but finally succumbed after two years.

CASE 22.—Mrs. W., a white female aged sixty years, was treated by her physician for fluid in the chest. She had rapid refilling after each tapping. The fluid was hemorrhagic in nature and microscopically showed many sheets of palisade-arranged cells which were uniformly round and oval, foamy with vesicular nuclei. A diagnosis of pleural endothelioma was made and confirmed by autopsy later.

CASE 23.—Mrs. E. W., a white female aged fifty-eight years, was admitted to the Sydenham Hospital on Jan. 10, 1933, with abdominal ascites. She had been laparotomized at another hospital several months previously, and was admitted to this institution for paracentesis. A paracentesis resulted in 1,800 c.c. of straw-colored fluid which showed numerous epithelial cells in solid clumps and in acinous formation. Many mitotic figures were seen. A diagnosis of epithelial malignancy was made and confirmed by the previous laparotomy.

CASE 24.—Mrs. A. M., a white female aged sixty-two years, was admitted to the Sydenham Hospital on Sept. 26, 1932, with marked anemia, some jaundice and a history of loss of twenty-five pounds over a period of three months. A calculus was found in the left kidney and also a lumbar arthritis was discovered. There was an abdominal ascites which at paracentesis yielded a straw-colored fluid. Microscopic study failed to reveal any neoplastic cells. At operation there was a chronic cholecystitis with stones in the cystic and common ducts. She proceeded to make an uneventful recovery.

CASE 25.—Mrs. D. G., a white female aged thirty-seven years, was admitted to the Sydenham Hospital on June 9, 1931, for hysterectomy for a fibroid uterus. She had an uneventful recovery and was discharged, but returned on Dec. 8, 1932, with ascites and a rup-

tured duodenal ulcer. The abdominal fluid was examined microscopically and a diagnosis of an epithelial malignancy was made, but not confirmed at operation.

CASE 26.—Mrs. M. D., a white female aged forty-one years, was admitted to the Sydenham Hospital on Jan. 27, 1933, with a history of illness for four months, her symptoms simulating an acute upper respiratory infection. Her condition became progressively worse and the loss of weight became marked. She developed a large amount of fluid in the right pleural cavity. A thoracentesis was productive of 600 c.c. of bloody fluid, and three days later 400 c.c. more were removed. Microscopic study of the sediment showed an epithelial neoplasm. The radiographs gave a picture of primary growth of the lower lobe of the right lung.

SUMMARY

1. There is presented a modification of the present-day methods of utilizing the sediment in exudates as tissues for histologic study. This modification causes less stress on the chemical and physical properties of the cells, thus preventing their distortion and preserving them for diagnosis.

2. Twenty-five cases, carefully checked up by subsequent histories, operations, and autopsies whenever possible are added to the literature.

3. The authors feel that the study of fluid sediments from a histopathologic viewpoint can prove a very valuable adjunct to clinical diagnosis, in addition to pointing the way for therapy in doubtful cases.

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565 MANHATTAN AVENUE.

SIMPLE METHOD OF DRYING PIPETTES*

VIRGINIA M. WALKER, MOBERLY, MO.

A SIMPLE and inexpensive device for drying pipettes, where a laboratory does not have access to compressed air, may be made by using an ordinary air pump for automobile tires.

This pump may be fastened securely in an upright position on the worktable near the sink. The rubber tubing is placed over the end of the pipette, and two or three pressures of air will be sufficient to dry any pipette of ordinary size.

If one has a properly fitting adapter for a Luer type syringe, it may be placed on the end of the rubber tubing and hypodermic needles dried in the same way.

*From the Woodland Hospital.

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THE PURIFICATION OF GUM DAMMAR FOR USE AS A MOUNTING MEDIA*

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GUM dammar, when in solution with the various well-known solvents, has always been considered an ideal mount-material affording better definition of delicate detail than the commonly used Canada balsam, due to its light colored solution and low refractive index. In spite of these desirable qualities it has been little used for permanent mounts because it forms either macroscopic or microscopic granules which causes confusion when examined at a later date. It has a tendency to become acid, thereby affecting nuclear stains. The solution, upon standing, becomes cloudy and the dried film when deposited from a benzol or xylol solution is hard and brittle. No wonder that this resin has been relegated to the scrap heap.

Gum dammar has been used in the varnish and lacquer industries for many years and while considered a very desirable resin, it presented many problems before it could be used to give a satisfactory film. Varnishes were first made by dissolving the gum in an appropriate solvent and sold to the trade as a cheap substitute. Under these conditions the dried film had a tendency to become white, powdery, and brittle. The films would not withstand shock or friction which made them undesirable.

Chemically, gum dammar has been found to consist of dammarolic acid, alpha dammar resene, and beta dammar resene. The last named constituent being a waxy substance with a low tensile strength is less soluble in the ordinary solvents and has a tendency to crystallize out during the evaporation of the solvent. Many processes have been developed for separating this substance from the other two constituents,† which rely on the difference in solubility between beta dammar and alpha dammar combined with dammarolic acid. The resulting material after the separation has remarkable properties as a varnish gum.

The author has had occasion to prepare many dammar-xylol solutions and has always been confronted with the above difficulties. By taking advantage of the method of treatment used in the lacquer industry, the resulting resin gave very desirable films, had none of the defects found in the raw gum solution, and still retained its excellent optical properties.

*Received for publication, March 2, 1932.

†British Patent No. 209,701; March 15, 1928. German Patent No. 503,615; August 30, 1927.

METHOD OF PREPARATION

A good grade of clear Indian or Malayan dammar is put into solution in the following combination of solvents:

Dammar	450 Grams
Ethyl acetate	57 c.c.
Acetone	57 c.c.
Benzol	266 c.c.
Methyl alcohol	285 c.c.

First mix the solvents thoroughly and then add the gum crushed to about the size of a pea. With occasional shaking during a period of four or five days, the resin will be completely dissolved, leaving all débris and a white slimy stringy mass, the beta dammar, in the bottom of the flask. Allow this to settle and then filter the clear filtrate directly into an evaporating dish. Evaporate on a water-bath until the resin is hard and while still hot add an equal volume of xylol. The addition of the xylol to the hot or melted gum facilitates a solution readily. At this stage sodium bicarbonate in excess is added and the solution is stirred by a mechanical stirrer for a period of twenty-four hours to neutralize any free acidity. The excess sodium bicarbonate is allowed to settle and the clear filtrate is either decanted or poured through a filter. The gum solution is now evaporated on the water-bath to any consistency desired.

In the event that the heating process has darkened the resin solution, decolorizing may be effected by adding activated charcoal to the thin xylol solution, heating for several hours on the water-bath, replacing the evaporated solvent and again filtering.

The advantages of this process are that granules are not formed in the dried film, there is no acidity developed, the solution remains perfectly clear, the optical properties of the gum are not altered, and the resin does not dry too quickly, becoming hard and brittle.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

BILIRUBIN, Concentration and Precipitation of, in the Gall Bladder and Bile Ducts, Elton, N. W., and Deutsch, E. *Arch. Path.* 15: 818, 1933.

The purpose of this study was to determine whether any physiologic basis existed for the precipitation of bilirubin on preexisting nuclei.

In a study of the concentration of bilirubin in the gallbladders and bile ducts of twenty-three cats and nine dogs the most dilute specimens were obtained, not from the hepatic ducts after cholecystectomy, but from the gallbladders as they were refilling after postprandial evacuation, and the most concentrated specimens were secured from gallbladders after the animals had fasted for from twenty-four to one hundred and forty-four hours. Hepatic duct bile after cholecystectomy invariably yielded a higher bilirubin content and icteric index than the early refillings from the gallbladders.

The factor of bilirubin concentration for dogs was found to be 50.9, for cats, 40.4 and for both animals together 83.3. According to the work of Kalk and Schondube, the factor for either of these animals is appreciably less than that found for man.

The bilirubin icteric index ratio averages lower for gallbladder bile than for bile obtained from the hepatic ducts after cholecystectomy in these animals.

Correlation of the refractive index with the quantitative bilirubin or the color intensity of bile indicates that a critical concentration exists at which bilirubin tends to precipitate out of solution. When this critical concentration is exceeded, the density of bile at once attains its maximum, being constant thereafter in spite of continued concentration. These findings indicate that a limit of crystalloid concentration exists, affecting bilirubin, bile acid derivatives and cholesterol, and effective when the critical concentration is exceeded, and that their further concentration can be only as suspended colloids or undissolved crystals.

Studies on the concentration of bile during fasting showed that the maximum level of bilirubin is usually attained in the gallbladder within twenty-four hours, and that after this interval hepatic bile leaks into the duodenum. The density was found not to increase, even after six days of fasting, above the value attained in twenty-four hours.

The aqueous (direct) van den Bergh reaction on fresh bile simulated any of the various types of reactions described for icteric serums, including immediate blue violet and red reactions, slow and delayed reactions and the negative reaction.

Since gallstones are seldom found in the cat and the dog, the physiologic precipitation of bilirubin during its concentration in the normal gallbladders of these animals cannot be regarded as the exciting cause in the origin of biliary calculi, but affords a physiologic basis for deposition of the pigment on preexisting nuclei.

AGGLUTINATION, H & O following Typhoid Vaccination and in an Unselected Group of Individuals, Dulaney, A. D., Wickle, W. T., Stewart, R. L., Bayfield, J. D., Walker, J. K., and Preacher, A. B. *J. Immunol.* 24: 229, 1933.

From a study of 90 serums from vaccinated (TAB) individuals and 100 unselected Wassermann serums it is concluded that:

1. Typhoid vaccination stimulates the production of H agglutinins to high titers. O agglutinins are also stimulated, but show much lower titers.
2. O agglutination in serum dilutions of 1:100 was demonstrated in 26 per cent of all vaccinated serums and 11 per cent of a random group of individuals.
3. If O agglutination is to have diagnostic value in typhoid fever, higher dilutions of serum than those recommended by Felix (1:100) must be employed. The authors recommend dilutions of 1:500.

FECES, A Convenient Specimen Container, Frobisher, M. Am. J. Pub. Health 23: 851, 1933.

The outfit consists simply of a 2-ounce, wide-mouth glass bottle with a cork stopper. A wooden tongue blade is cut into halves and one end of each piece is whittled to a sharp, flat point which is thrust firmly into a cut in the narrow end of the cork, the cut in the cork having previously been made with a paring knife. The cork is then fitted into the bottle and the whole sterilized in the hot air oven at 170 C. for two hours.

A good grade of cork or a rubber stopper must be used in order to avoid leakage if pressure develops. If a preserving solution is used, sterilization may be accomplished in steam.

A slightly more expensive modification of this outfit may be made from sheet metal. The wooden paddle is replaced by a piece of metal, and this may be fastened with a few drops of solder (necessitating steam sterilization) to the inside of a metal clamp or screw cover, very much in the same manner as small brushes are supplied in cans of certain brands of automobile "touch up" paint. The possibility of bactericidal or bacteriostatic effects due to the metal must be taken into consideration before adopting such a device. Much will depend on the distance, time and temperatures involved in transporting the material to the laboratory. Both types of paddle can be used more than once. Metal paddles can be made spoon-shaped to facilitate the collection of liquid material.

In collecting the feces specimen the spatula always remains with the bottle, it is not touched by anyone and no disposal or esthetic problem arises.

TUBERCULOSIS: A Technique for Estimating the Number of Tubercle Bacilli in Sputum, Hughes, J. Am. Rev. Tuberc. 28: 279, 1933.

Preparation of Specimen and Smear.—One cubic centimeter of a 10 per cent solution of sodium hydroxide is pipetted into a test tube of 10 c.c. capacity, and 2 c.c. of sputum is added, along with three glass beads. The mouth of the test tube is flamed, and stoppered with a cork. The tube is then placed in a shaking machine in a horizontal position, and agitated. Because of the viscosity of sputum, pieces of ordinary glass tubing, 4 mm. in diameter and 25 cm. in length and graduated by a mark with a file to deliver 2 c.c., have been used instead of the usual type of pipette. Sputum can be readily drawn up into such a pipette by means of a Luer syringe attached to it by rubber tubing.

After being digested and shaken for thirty minutes the specimen is removed from the shaking machine. The following technic is used in making smears:

An area of 400 sq. mm. is marked off on the end of a one-inch microscopic slide by drawing with a diamond pencil a line 1.5 cm. from one end. This measurement introduces an error of less than 0.5 per cent. The slide is then cleansed by being placed overnight in a solution of alcohol, 97 c.c., and acetic acid, 3 c.c. By means of a standard loop, 3 mm. in diameter, made of platinum wire 0.4 mm. in thickness, sold by dealers in laboratory equipment, one loopful of serum used to fix the bacilli to the slide and one loopful of the digested sputum are mixed together on the slide, and are then spread in a uniform smear over the marked-off area of 400 sq. mm. The smear is dried in air, fixed by heat and stained by the usual Ziehl-Neelsen technic. Ten scattered oil-immersion fields are examined and the average number of bacilli per field is estimated. This average is then corrected for the dilution of the sputum by sodium hydroxide by multiplying the number of bacilli seen per field by the factor 1.5.

Since, in the present method, the 3 mm. loop used in making the smear delivers approximately 3.5 c.mm. of treated sputum, it is possible to estimate the number of bacilli in 1 c.mm. of sputum after calculating the average number of bacilli per microscopic field and the number of fields in the smear. This may be expressed by the following equation:

$$\text{Average number of bacilli seen per microscopic field} \times \frac{\text{Number of microscopic fields of smear}}{3.5} = \frac{\text{Number of bacilli in 1 c.mm. of untreated sputum}}{3.5}$$

In the microscope used for this work there were 25,000 fields in the area of the smear, but this figure will vary depending upon the size of the oil-immersion field of different microscopes. It can be readily determined for any microscope by calculating the diameter of the oil-immersion field by focusing on the rulings of a hemocytometer that are 0.05 mm. apart. In doing this it is necessary to use a No. 1 or No. 2 cover slip to cover the rulings, because the oil-immersion lens cannot be used with the heavier cover-slip provided with the hemocytometer.

By working out the equation for estimating the number of bacilli in 1 c.mm. the factor 10,714 was obtained, which, when multiplied by the average number of bacilli per oil-immersion field, gave the number of bacilli in 1 c.mm. of sputum.

RENAL FUNCTION: In Persons With One Kidney, Ellis, L. B., and Weiss, S. *Am. J. M. Sc.* 186: 242, 1933.

The renal function of 12 patients who had undergone unilateral nephrectomy was studied by urea and creatinin clearance, concentration and phenolsulphonephthalein tests. Nine of the patients were quite healthy, 2 had pyonephrosis of the remaining kidney and 1 had hypertension.

The 2 cases which were studied immediately after operation showed diminished values for the clearance and concentration tests. The tests on 7 other uncomplicated cases were normal in 2, and one or more of the tests were slightly abnormal in 5. The 3 patients with complications had marked impairment of renal function.

In the absence of complications, the renal function of persons with one kidney is not only adequate, but possesses a reserve capacity as well.

The observations confirm the value of urea and creatinin clearance tests in detecting early and quantitative reductions in glomerular function and of the concentration test as a measure of tubular reabsorptive power.

RENAL FUNCTION: Normal Variations in Renal Function Tests, etc., Ellis, L. B., and Weiss, S. *Am. J. M. Sc.* 186: 223, 1933.

On 12 normal subjects urea and creatinin clearance tests were performed and on 5 subjects dilution concentration tests were carried out. These studies were made to show the normal variations of each test and the effect of changes in one test on the others, and to correlate the results with the physiologic functions of the kidney which they measure.

The creatinin clearance test probably gives a close estimate of the absolute degree of glomerular filtration and the urea clearance provides a relative index of this function. Clinically, either test may be used.

The ability of the renal tubules to reabsorb fluid is best measured by a concentration test.

To attain as adequate an estimate as is possible by laboratory methods regarding the state of renal function, the tests mentioned together with an ordinary examination of the urine will suffice.

At best the information gained from these procedures merely supplements that obtained by the actual clinical observation of the patient.

PELLAGRA: A Chemical Peculiarity of Pellagra Blood (Rapid Iodin Decolorization), Campbell, C. H. *Am. J. M. Sc.* 186: 266, 1933.

Pellagrous blood reduces iodine solutions at a constantly greater rate than that of any other blood thus far examined. This phenomenon is made the basis of a test which promises to be of clinical value in the diagnosis of pellagra. Many pathologic conditions have not been examined, and it is possible that further work will disclose that other conditions simulating pellagra may give positive reactions. However, the phenomenon of increased rate of iodine decolorization by the erythrocytes in pellagrous blood may lead to fruitful information bearing on the etiology of pellagra, certain entities of avitaminosis and malnutrition.

Reagents.—

Alcohol-ether Mixture: A mixture of 3 volumes of 95 per cent ethyl alcohol and 4 volumes of ether (commercial or anesthetic ether may be used; chemically pure ether has not been tried).

Iodine Solution: Accurately prepared Lugol's solution (5 per cent iodine in 10 per cent potassium iodide).

Color Standards: Prepare a 3 per cent potassium dichromate solution and from this make the following dilutions: 1 to 5, 1 to 10, 1 to 20, 1 to 40, 1 to 80, 1 to 120, 1 to 240. Place these standards in a series of test tubes of the same internal diameter.

Procedure.—

Liquid Petrolatum. Five cubic centimeter samples of normal or nonpellagrous (control) and of suspected pellagrous venous blood are withdrawn and quickly introduced to 15 c.c. centrifuge tubes containing 5 c.c. of liquid petrolatum. The time of collection of each specimen must be recorded. As each sample is collected, the tube is stoppered and shaken very vigorously until the blood is defibrinated. This may best be accomplished by striking the stoppered end of the tube against the palm of the opposite hand. The specimens are then allowed to stand for thirty to forty-five minutes, after which they are again vigorously shaken and centrifuged at 1,000 revolutions per minute for 5 minutes. Before the second shaking it is advisable to remove the stoppers momentarily to permit the entrance of air for adequate oxygenation. When the tubes are removed from the centrifuge three distinct layers may be distinguished, namely, an upper layer of fibrin and oil, a middle layer of serum and a lower layer of erythrocytes, leucocytes, etc., which the author shall designate the erythrocytic layer. If any sample has not been properly defibrinated clots will be present in the lower layer. Such samples should be discarded. One cubic centimeter samples of the erythrocytic layers are now transferred by means of serum pipettes to test tubes of the same diameter as those of the standards. In the removal of these samples from the erythrocytic layer, care must be taken that no oil, fibrin or serum enters the pipette by capillary action as it passes through the two upper layers.

Exactly one hour after taking the venous blood specimen, add to the corresponding erythrocytic sample, slowly and without shaking, 5 c.c. of the alcohol-ether mixture and stopper the tube tightly. After the alcohol-ether mixture has been added to all samples, let them stand for approximately 6 hours at room temperature (25° to 30° C.). At the end of this time add in rapid succession to each tube by means of an accurate micro pipette (Folin micro blood pipette or Kahn serologic pipette) a 0.1 c.c. portion of the iodine solution. Mix gently and replace the stoppers.

BLOOD: A Study of a Lymphocytic Hemogram, Reich, C., and Reich, E. Am. J. M. Sc. 186: 278, 1933.

A lymphocytic hemogram has been studied, using basophilia of the cytoplasm as a criterion of the age of the lymphocytes.

The lymphocytic formula is stable in health, but is more labile than the Schilling index, and cannot be used to gauge the severity of an infection. Its shift to the left in infections nevertheless indicates that the lymphocytes take an active part in combating these conditions.

The lability of the lymphocytic index makes it valuable in detecting minor infections which do not disturb the more stable Schilling index.

The lymphocytic index is superior to the Schilling in guarding the progress of convalescence, and a patient should not be regarded as entirely well until the lymphocytic formula has returned approximately to normal.

Analysis of the experimental data shows that there is a fairly constant lymphocytic formula for normals. For adults this can be expressed as Y forms, 5 per cent; M forms, 50 per cent; O forms, 45 per cent. Variations occur in this formula under different conditions, and we can, therefore, speak of a shift to the left or to the right of the lymphocytic index, depending upon whether the number of younger forms is greater or less than normal.

A comparison with the Schilling index in each case shows that the two do not run parallel. The lymphocytic index is not as stable as the Schilling and will shift to the left just as easily in a mild as in an acute infection. Even though the lymphocytic formula cannot be used to gauge the severity of an infection, its shift to the left in these conditions nevertheless indicates that lymphocytes take an active part in combating bacterial invasion. This is seen by the increase in Y and M forms and the decrease in O forms in practically all cases of infection.

The lability of the lymphocytic hemogram makes it valuable in the detection of minor infections which do not disturb the more stable Schilling index. Interesting and confirmatory evidence for using basophilia as a criterion of the age of lymphocytes is seen in the study of the lymphatic leucemias. The marked increase in the proportion of Y and M forms in these cases represents the well-known hyperactivity of the lymphatic system in these diseases. In the myeloid leucemias there is also an increase in lymphocytic activity and a shift of the index to the left.

It is also of interest to note that, according to the lymphocytic index, most patients are not entirely well on being discharged from the hospital and that the period of convalescence is probably much longer than usually expected. A patient should not be regarded as entirely recovered from an infection until the lymphocytic formula has returned to normal.

Technic.—White and differential counts were done for each observation. The total white counts were done with carefully standardized pipettes, and the films were made on slides, care being taken to keep them "rim free." The stain used was Wiseman's Wright-Giemsa. An average of 200 leucocytes was counted and the lymphocytes were divided into three classes: Y (young), M (medium), and O (old) forms, according to the degree of basophilia of their cytoplasm. The Y forms have a deep blue cytoplasm, the M forms moderately blue and the O forms are faintly blue or colorless, and usually show azure granules in the cytoplasm, although the M forms occasionally show them too. A little practice with the above technic will make the three divisions clear. It is necessary, however, that the lymphocytes be examined in those parts of the film in which the red cells do not overlie, as in the thick portions of the film the lymphocytes are likely to overstain. The total number of lymphocytes is noted as well as the number of Y, M and O forms, and the percentages of each are then easily calculated, the procedure being similar to that followed in enumerating the different forms of polymorphonuclears when doing the Schilling index. When the total number of lymphocytes is low it is best to count 300 to 500 white cells to insure more accurate results. The polymorphonuclear leucocytes were tabulated according to the Schilling method in each instance in order to compare the granulocytic and lymphocytic indices.

RENAL FUNCTION: The Fractional Phenolsulphonephthalein Test in Bright's Disease, Chapman, E. M., and Walsted, J. A. *Am. J. M. Sc.* 186: 223, 1933.

The fractional method of estimating the elimination of phenolsulphonephthalein (15, 30, 60 and 120 minutes) has been studied in the past two years in 20 normal subjects and a large number of patients with suspected renal disease.

The authors have found that in Bright's disease the fractional test may show evidence of impaired renal function when the test as usually done, with hourly collections, is interpreted as normal.

This fractional test is quite as informative as the urea clearance test and reflects the diminishing function in progressive kidney disease.

Since it is easier to perform, it is the method of choice for routine clinical work.

BLOOD: The Neutrophil in Pernicious Anemia, Heck, F. J., and Watkins, C. H. *Am. J. Clin. Path.* 3: 263, 1933.

Fifty cases of pernicious anemia, in all of which there was achlorhydria, have been studied with reference to the lobulation of the neutrophil. Increased segmentation in the neutrophil is the rule, although cases do occur in which there is a left shift and absence of the hypersegmented neutrophil.

In three cases in which there were morphologic changes of pernicious anemia in the presence of adequate quantities of free hydrochloric acid, but in which other characteristic symptoms of pernicious anemia were absent, no essential differences could be found from the former group.

There is no invariably pathognomonic blood picture of pernicious anemia, since the same changes may be demonstrated in sprue and occasionally in an indeterminate group of anemias.

SPIROCHETA PALLIDA. A New Method of Staining (Su Di Un Nuovo Metodo Di Colorazione Del Treponema Pallidum), Pinetti, D. Diagn. E. Techn. Di Lab. 4: 205, 1933.

Pinetti has had excellent results with the modification of Romby's method which is described below:

Reagents.—

A. Acetic Copper Solution:

Chloride of copper	gr. (gm?) 1
Acetate of copper	gr. (gm?) 1
Acetic acid, glacial	15 c.c.

Dissolve the copper salts "as far as possible" in cold distilled water (volume not stated) with frequent shaking and allow to stand 30 minutes. Then heat to boiling and allow to cool, when the acetic acid is added. Filter twice through filter paper and keep in a well-stoppered dark-colored bottle.

If turbidity develops in the solution on standing the clear, emerald-green color can be restored by the addition of a few drops of glacial acetic acid.

B. Fixing Solution:

Osmic acid	gr. (gm?) 1
Distilled water	400 c.c.

C. Staining Solution:

Freshly prepared carbolfuchsin (Ziehl Neelson).

Method.—

1. Cover the smear with a freshly prepared mixture of equal parts of A and B and allow to act for ten minutes. If necessary, add more solution so that the entire surface of the slide remains covered.

2. Pour off the solution and drain.

3. Without washing, cover with the stain and allow to act for five minutes.

In a properly stained slide the treponema are seen as violet red on a rose-colored background. Spirilla, and other organisms are deep red. A deeply stained background indicates an error of technic or in the preparation of the reagents.

Equally good results may be had by using, instead of carbolfuchsin, the stain below:

Victoria blue (Gruhler, R4)	gr. (gm?) 1
Thymol	gr. (gm?) 0.20
Methyl alcohol	30 c.c.
Distilled water	70 c.c.

With this stain, applied as above described, the treponema are an intense blue black, their delicate structure being exceptionally well seen.

The background is clear, precipitate being rare.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Red Blood Cell Diameters*

See Editorial in this issue.

Manual of Urology†

THE purpose of this manual is to present the fundamentals of urology so that they may be easily grasped by the student approaching this subject.

The arrangement of the subject matter is designed to emphasize the etiological factors concerning the disease in question in the discussion of which, as the book is intended for the student, only generally recognized conclusions are presented.

To those who are desirous of obtaining a fundamental groundwork in urology upon which to erect a comprehensive knowledge of the subject this book can be recommended as eminently suitable for the purpose.

Blood Pictures‡

THIS small volume, addressed to the practitioner, has for its purpose the presentation of simpler methods of hematological examination, followed by brief résumés of the blood pictures in disease and their interpretation.

The book is succinct in style and serves, as the title indicates, as an introduction to the subject.

This, the third edition, has been revised in keeping with the advances made in this subject.

The Common Cold§

THIS, the Eighth Volume of the Annals of The Pickett-Thomson Research Laboratory, is devoted to a comprehensive discussion of the common cold with special reference to the part played by streptococci, pneumococci, and other organisms.

The publications from this laboratory need no introduction and it is almost unnecessary to say that the present volume is in keeping with and worthy of the standard set by its predecessors.

As is now well recognized, the common cold constitutes one of the most—if not the most—important factors in the morbidity and mortality rates of the civilized world and, because of its economic importance as a cause of physical wastage and inefficiency, has become the subject of concerted and intensive study.

*Red Blood Cell Diameters. By Cecil Price-Jones M.B. (Lond.) Humphrey Milford, Oxford University Press, New York Branch, 1933. pp. 82, cloth.

†Manual of Urology. By R. M. LeCompte M.D., Professor of Urology, Georgetown University Medical School. Cloth, 317 pages, 46 figures. William Wood & Co., Baltimore.

‡Blood Pictures: An Introduction to Clinical Hematology. By Cecil Price-Jones. Cloth, 72 pages, 5 colored plates. William Wood & Co., Baltimore.

§The Common Cold. By David Thomson and Robert Thomson, The Pickett-Thomson Research Laboratory, St. Paul's Hospital, London. Cloth, pp. 738, 51 plates. The Williams & Wilkins Co., Baltimore.

In three cases in which there were morphologic changes of pernicious anemia in the presence of adequate quantities of free hydrochloric acid, but in which other characteristic symptoms of pernicious anemia were absent, no essential differences could be found from the former group.

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EDITORIAL

Erythrocyte Diameters

FOLLOWING the development of the modern methods in the study and treatment of pernicious anemia, interest in erythrocytology has been widely awakened. Smears that formerly would have been classed as indicative of secondary anemia have occasionally been found to be those of primary anemia. Smears that most pathologists would interpret as characteristic of pernicious anemia have been found to occur in patients that do not have primary anemia. There appears to be a distinct group of this second class. Not infrequently one observes blood smears with clear-cut macrocytosis, with or without anemia, which are definitely not associated with pernicious anemia. As a consequence the measurement of erythrocyte diameters not infrequently becomes a matter of diagnostic laboratory routine.

Attempts to measure red cell diameters are by no means new. While some of the earlier methods were crude and the conclusions were not altogether cor-

The present volume, because of its comprehensive review and summary of the work thus far done, ranks as a study of first importance and may well be read by all who are interested in this subject.

Starting with a review of the structure and physiology of the nose and nasal sinuses, the researches concerned with the bacteriological flora of the healthy nose, nasopharynx, mouth, trachea, bronchi, and lung alveoli are next described as determined not only by the investigations of the authors, but also as reported in the literature of the world.

The plurality or nonplurality of colds as a disease, the relationship of the common cold to influenza, and the noncontagious colds are next considered, followed by exhaustive studies of the rôle of various specific organisms as causative agents. Subsequent chapters discuss various predisposing factors, complications, treatment and prevention so that, all in all, the volume is practically encyclopedic in its handling of the subject.

The authors' views deserve careful consideration and can only briefly be summarized here.

They are not convinced that the common cold is a definite single disease but regard it as a group of diseases, arising from varied causes. They recognize, hence, bacterial colds, allergic colds, and possibly virus colds. While not unmindful of the work done concerning the part played by a filter-passing virus as an etiological agent of colds, they believe that to regard all colds as so caused is carrying the theory too far and casting aside without warrant the work proving the important relation of bacteria.

They are convinced that the commonest bacterial variety of cold is the pneumococcal, followed by the streptococcal and streptopneumococcal. Of importance, also, are the colds due to invasion by Pfeiffer's bacillus, and *M. catarrhalis*, other bacterial colds being infrequent. They comment, also, that it has not yet been shown that filtrable forms of these bacteria may not be the answer to the filter-passing virus theory.

It is impossible, in the space available for review, to convey more than a mere outline of the ground covered by this most comprehensive and valuable report which should be available to all laboratory workers and may well be read with profit by the physician at large.

The format and the excellent microphotographic illustrations will be familiar to those who have seen the previous volumes of this series.

As this reviewer has previously said, it seems a pity that these volumes of such value should not have a more durable covering than paper, for they are sure to be well read and frequently consulted.

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Attempts to measure red cell diameters are by no means new. While some of the earlier methods were crude and the conclusions were not altogether cor-

rect, it is indeed surprising how accurate they were, considering the methods available. Cecil Price-Jones in his very informative monograph on *Red Blood Cell Diameters* reviews the history of this phase of hemocytometry in conjunction with his presentation of his own very detailed studies of the measurements of red cells in disease and in health.

As long ago as 1673, Leeuwenhoek attempted measurement by the very crude method of placing minute grains of sand in a row, one inch long, all grains being as nearly as possible the same size. By counting the number of grains in contact to make an inch, he was able to estimate the diameter of one grain in terms of fraction of an inch. With this grain for comparison he attempted to measure red cell diameters. Later investigators substituted lycopodium spores with a mean diameter of $\frac{1}{940}$ of an inch for the sand grains.

Jurin in 1718 twisted fibers of silk or hair around a pin, with the successive twists in close contact; counted, under the microscope, the number of coils per unit measure, thereby determining the mean thickness of the hair. A segment of the hair was then compared with erythrocytes. This method was not altogether satisfactory since the hairs and fibers varied in thickness. He calculated the erythrocyte diameters in one series as averaging 7.75 microns, and in another series 12.5 microns.

de Senae in 1749 compared red cell diameters with a standard line, concluding, first that they measured 7.22 microns, later 7.69. Hewson in his famous work on the blood (1770) makes no definite estimate of the size of the erythrocytes but does state that the size varies in different animals and sometimes in the same animal, and again at different periods of life. This was particularly true in the chick.

Prevost and Dumas (1821) employed binocular vision, observing the cells through the microscope with one eye while their image was superimposed upon a scale placed next to the microscope and seen with the other eye, with accommodation relaxed. They estimated the mean diameter as 6.6 micra. Young in 1823 attempted measurement by comparing the luminous halo surrounding the erythrocytes with that surrounding other objects of known diameter, and of approximately the same size, concluding that the diameter of the human red corpuscle was between 5 and 6 microns.

Lister, the father of Lord Lister, and his associate Hodgkin, using Amici's camera lucida (1827) selected 8 microns as the correct size. Gulliver in 1846, using a micrometer eyepiece found the diameter of human red cells to average 7.61 microns. He found that of the elephant, 9.1; the whale 8.03; the musk deer 2.02; the cat 5.6; and dog 7.05.

Price-Jones using a projection apparatus adjusted for a magnification of 1,000 (1910) measured the resultant images with a millimeter scale, recording these figures directly in terms of microns. He found the mean diameter in healthy persons based upon measurement of 10,000 cells to be 7.21 microns, with a mean range from 6.96 to 7.49 (1922). In a later series based upon measurement of 50,000 red cells, the normal diameters were found to range from 4.75 to 9.50 with a mean diameter of 7.202, a standard deviation of 0.487 and a coefficient of variation of 6.325 per cent.

In measuring erythrocyte diameters Price-Jones emphasizes the need for a standard and unvarying technic in preparation. His technic is briefly as follows: A thin spread, air dried, fixed and stained with Jenner stain for two minutes; washing with distilled water and drying, followed by a two-minute super staining with weak aqueous solution of eosin. Deviations from this standard procedure were found to sometimes produce changes in the mean diameters. He prefers counts of 500 cells, with a minimal number compatible with reasonable accuracy of 200. Largest and smallest diameters are taken for each cell, the diameter value of the cell being the average of these two. The arithmetic mean, the standard deviation, and coefficient of variation are then calculated mathematically.¹

Price-Jones finds, even in normal individuals that there is a distinct diurnal variation in red cell diameters, probably dependent upon the hydrogen ion concentration. There is a gradual increase in size during the day, with a diminution during sleep. The variation may amount sometimes to as much as 0.6 microns. Gentle exercise has no apparent influence but violent exercise increases diameters. Nevertheless rest in bed throughout twenty-four-hour periods does not abolish the diurnal variations. The increase in size following violent exercise may amount to as much as 0.6 microns.

The observation that local cyanosis increases diameters, anywhere up to 0.4 microns, emphasizes the need for obtaining specimens without the use of a tourniquet. Cells in venous blood are larger than those in arterial blood.

These variations in size appear all to be due to changes in hydrogen ion concentration, the swelling being associated with a decrease of alkalinity. It has been shown both in vitro and in vivo that an increase in concentration of lactic acid or of carbon dioxide causes swelling. The washing of CO₂ out of the blood by forced breathing was found to diminish the mean diameter by 0.5 microns. Discontinuance of forced breathing brought a rapid restoration to normal diameters. There is evidence that the blood is more alkaline during sleep than in the daytime.

Pulmonary emphysema, especially when associated with some degree of cyanosis is accompanied by macrocytosis, the mean diameters ranging from 7.33 to 8.17 with a mean diameter for the series of 7.69. On the basis of the same considerations it seems entirely possible that certain chemical poisonings should produce a macrocytic anemia suggestive on microscopic examination of pernicious anemia, but in no way connected with the latter disease. Indeed Beek² has described a blood picture following low grade chronic carbon monoxide poisoning, almost indistinguishable from that of pernicious anemia.

The mean diameter following hemorrhage is lower than the mean for healthy persons, averaging 6.879 with a range from 6.3 to 7.225. The distribution curve is shifted to the left of the healthy curve. There is greater variation in the curves, the coefficient of variation averaging 10 per cent, due presumably to the mixture of two types of cells, the mature red cells and smaller, newly formed cells which have been poured into the circulation as a result of sudden overstimulation of the marrow.

In pernicious anemia, Price-Jones found a variation in diameters from 3.75 to 13.0, with a mean diameter of 8.31, over one micron greater than the

average for healthy red cells. The coefficient of variation was as high as 16.3 per cent and the excess of large cells resulted in a megalocytosis of 40.4 per cent. Microcytosis was also present, in about 75 per cent of the cases.

The mean cells of normal persons show a curve of variation which is sharply pointed and quite symmetrical. That for pernicious anemia has a much broader base and is decidedly irregular, usually asymmetrical. This suggests a heterogeneous population of red cells. It seems probable that in the blood of pernicious anemia there are at least three classes of red cells: large cells derived from megaloblasts which have failed to mature due to the lack of the specific intrinsic liver factor; normal size cells from what bone marrow is still reacting normally; and small cells which owe their origin to the same causes which are active in simple acute hemorrhage, that is extra stimulation of bone marrow caused by abnormal destruction of erythrocytes. That these three classes actually exist is further suggested by the observation that the degree of anisocytosis varies directly with the degree of anemia, while the red cell diameters are independent of the degree of the anemia. It would appear that the disease is responsible for the macrocytosis and is associated with the absence of the intrinsic liver factor, but that the presence of microcytes is directly due to bone marrow stimulation resulting from the anemia. Following liver treatment the macrocytosis becomes less pronounced. Indeed in many cases the mean diameter returns to normal.

The mean diameter in microcytic anemia, the chlorotic anemia occurring particularly in women during the child-bearing period and often associated with gastric subacidity or anaecidity, was found by Price-Jones to be 6.48 microns with a range from 6.2 to 6.7 microns.

The observations on red cell diameters reviewed above indicate not only the large amount of work which has been done in this field but also the possibilities for future contributions which may be made by those who in their routine work will recognize the apparent slight deviations from normal which will bear further quantitative study and which may lead to a clearer understanding of the subject.

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—W. T. V.

Erratum

In the article by Drs. Macht and Paulson *Phytopharmacology of Stomach Washings in Various Digestive Disorders and Pernicious Anemia* published in the November, 1933, issue, page 164, line 32, the percentage should read 86 instead of 6.

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SURGICAL MAGGOTS IN THE TREATMENT OF INFECTED WOUNDS: RECENT APPARATUS AND METHODS IN MAGGOT PRODUCTION AND RESEARCH*

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A PREVIOUS article in this JOURNAL³ and a recent circular of the U. S. Bureau of Entomology⁴ describe the biology of the blow flies and the production of sterile maggots. The present discussion deals with some recent devices and methods which have been found useful in maggot culture and research.

Culture Bottles for Sterile Maggots.—Aseptic rearing of maggots is carried on best in small lots. The principal reason for this is that if contamination is detected the loss in discarding that lot is correspondingly less when a small culture is used. Lots containing from 700 to 1,000 seem to be preferable.

For a culture of that size a wide mouth specimen bottle, Fig. 1 B, makes a simple and useful container. Shoulders are almost absent in this type of bottle which facilitates the removal of the maggots and food at the time of implantation. This is a stock bottle 3 inches high, 1½ inches wide with a mouth opening of 1¼ inches. It is made of heavy glass and is quite inexpensive. Shell vials of similar dimensions have been found useful also, but as they do not appear to be carried in stock by supply houses they have to be made up by a glass blower. Satisfactory results have also been obtained with the Naples staining jar, Fig. 1 A. It is made of heavy glass and has a base which prevents

*From the Division of Insects Affecting Man and Animals, Bureau of Entomology, U. S. Department of Agriculture.

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tipping. It costs about the same as the shell vial, both of which are more expensive than the stock wide mouth bottle.*

When being prepared for use, the containers are plugged with gauze-covered cotton and sterilized in a hot-air oven at 150-160° C. for one hour. The food (described later) is then added and autoclaved. Wire letter baskets are convenient for holding the containers.

Rearing Cage for Brood Larvae.—To replace fly colonies when egg production begins to decline, a certain proportion of maggots must be reared through to the adult stage. This process is somewhat different from production of sterile maggots and requires a different container. The biology involved in maggot rearing is discussed in the earlier article.³ By far the most satis-

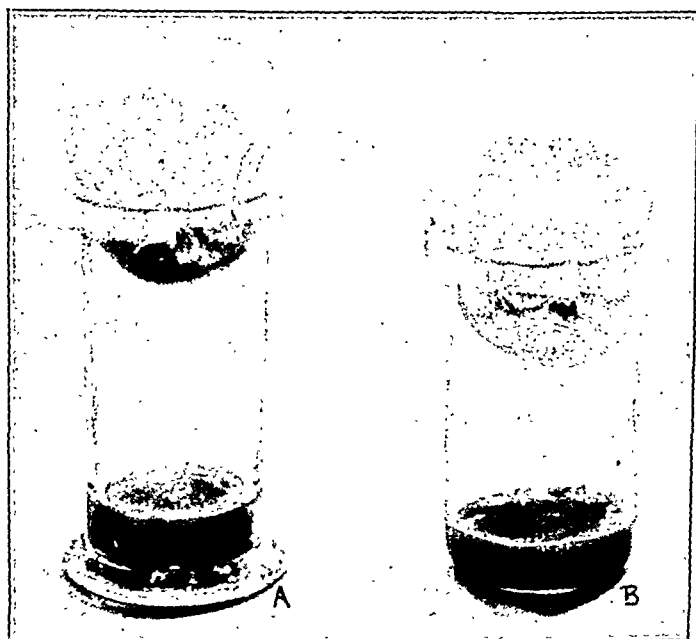


Fig. 1.—Food containers for culture of sterile maggots: A, Naples jar; B, specimen bottle.

factory cage for brood larvae, which this laboratory has used, is the double container illustrated in Fig. 2. It consists of an outer cage enclosing a food container and sand for pupation. It is a very simple device and easily obtainable. The outer cage may be a large glass beaker, a dressing jar, or preferably a commercial oiled-fiber can, as shown in the illustration, one-half gallon size, 5½ inches high and 5½ inches in diameter. The fiber cans are quite inexpensive and are useful for other purposes in the laboratory.

When the cage is being prepared for use, the material used for the migrating larvae (discussed later) is placed in the outer container. The inner one is then introduced. This may be similar to the outer one, but of much smaller size. If the fiber can is used, the inner one may be either a half-pint or pint size, preferably the former. With the meat and eggs added, this container is

*In case of uncertainty as to where to purchase these or other special articles mentioned, the Bureau of Entomology will, upon request, supply the necessary information.

placed within the cage and left uncovered. The outer cage, however, should be covered, to exclude parasites, which may become troublesome. Gases form during larval feeding and, if allowed to accumulate within the cage, tend to cause premature migration. Some ventilation is therefore necessary. A layer of cheesecloth makes a suitable covering, or, if observation is desired, perforated cellophane will be found convenient. If the fiber can is used, the cover may easily be attached by pressing the circular top out of the lid and using the rim as a collar to keep the cloth or cellophane in place.

With the closed container as described, the rearing of larvae for propagation requires but little attention. It should be noted, however, that an abundance of meat is necessary. Undernourished larvae will pupate but will develop into small flies with poor egg-laying capacity. The larvae will feed until full grown and then migrate without further care. Because of their compactness, these cages occupy little space within the incubator.

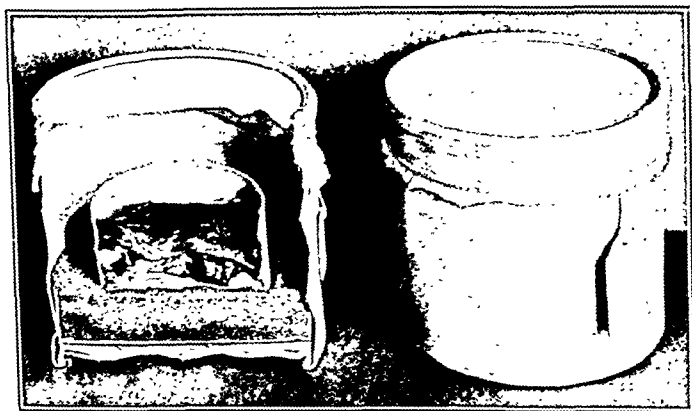


Fig. 2.—Rearing cage for brood larvae. One sectioned to show construction, and the other ready for use.

Material for Pupation of Larvae.—When the larvae begin migrating from their food, they must be provided with a suitable place for pupation. When this is supplied the maggots will enter at once; otherwise they will wander and possibly escape. Various materials, such as sand, shavings, sawdust, ground cork, and the folds of cheesecloth, have been used; but fine, clean sand is recommended by this laboratory. When sand is used it is very easy to recover pupae, when desired, by sifting. It is also available at most laboratories. About one inch of sand placed in the bottom of the outer container is sufficient.

The Water Fountain.—Where honey-yeast mixture is used as food for the adult flies, it will be noted that the mixture tends to dry out over hot weekends. Granulated sugar is an excellent substitute at such times; but when used, a supply of water must be provided. To avoid drowning of the flies, water is best supplied by means of a fountain. Several kinds have been tried, but the most satisfactory one is the type described by Miller.² It consists of a 50 c.c. beaker placed inverted upon a layer of filter paper in a Petri dish. The

lip of the beaker ensures a sufficient flow of water to keep the paper moist. Such a fountain will provide enough water for a cage of 150 flies for three or four days.

Determination of Number of Eggs.—In research in the field of maggot production it is sometimes necessary to know the number of eggs laid or to isolate a definite number. The eggs are usually laid in masses and remain attached to one another because of their mucoid surface. Estimating the number by the size of the clump has been found to be unreliable; on the other hand, counting individual eggs after separation is very tedious.

A method of determining the number by weight has been found satisfactory. A standard or constant was obtained by taking numerous egg-masses periodically from different colonies, weighing them, and separating and counting the eggs. In this way it was found that on the average 9.9 eggs weigh one milligram. This average may vary slightly from time to time and should be checked.

To make a determination, therefore, of the number of eggs in any clump it is necessary only to weigh the mass accurately and multiply the number of milligrams by the constant. An analytical balance should be used, preferably one of the modern type which permits rapid adjustment in milligrams. The eggs may be conveniently weighed upon a glass or tin foil plate.

Egg Viability Tests.—In the production of sterile maggots it will be found that a certain proportion of the eggs frequently fails to hatch. While this may not be important in routine maggot production, it is of significance in research, and determination of the percentage of failure under various conditions is sometimes necessary. The following method of determining viability has given good results.

Sterilized larval food is used in the test because the formation of molds or colonies of bacteria over the eggs prevents hatching. A few cubic centimeters of 1 per cent solution of plain agar in water is poured into the Naples jars or shell vials previously described. Small pieces of raw liver are then dropped into the agar. The pulverized liver mixture of Buchman and Blair¹ also gives good results. The containers are then plugged and autoclaved. The eggs used in the viability tests cannot be sterilized, as that would introduce another factor affecting hatch. A known number of eggs, probably 200 to 300, are placed without injury upon two thicknesses of wet sterile absorbent gauze about 2 inches square and transferred aseptically to the inside of the jar just above the food. Excess water should be previously drained off, otherwise the water contaminated by the unsterilized eggs might inoculate the food. The cultures are then placed in the incubator for hatching of the eggs.

When the larvae have fed for three days the food is usually sufficiently liquefied, and the maggots are large enough to strain out. Water is then poured into the jars and left for a few minutes, immersion separating the larvae rapidly from the food. If the maggots are numerous and active the water should be cold to reduce their activity while being counted. If their number is small, the food will not be sufficiently liquefied and warm water is preferable. The contents of the jars are then stirred, strained through a fine

wire sieve over a beaker, and transferred to a Petri dish. Estimation of number has been found especially unreliable, and determination of number by actual count appears essential. A hand counter has proved very useful in making counts.

Elimination of Odor.—Disagreeable odors develop readily during maggot production and research, but if sufficient care is taken they can be prevented from becoming noticeable in the laboratory. The exhaust draft recommended for the larval cabinet³ will prevent leakage of odors into the room. The ordinary type of household fan, however, placed within the cabinet to force air up a ventilator is usually not satisfactory. An exhaust fan installed on the ventilator line to draw off the odors is more preferable and is not expensive.

Residue from the feeding of brood larvae is especially offensive and should be promptly destroyed. Each laboratory will probably have certain facilities for waste removal. A very satisfactory method is to use a paper bag in a covered receptacle such as a large dressing jar or a can and to keep this in the larval cabinet or in a chemical hood. At the end of each day the bag is burned. An incinerator or steam generating plant is usually available to the laboratory.

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HYPOLUCOCYTIC ANGINA*

AN UNUSUAL FORM OF INFECTIOUS LEUCOPENIA

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THIS syndrome is clinically similar to agranulocytic angina in its sudden onset, high fever, prostration, and the presence of necrotic ulcerations of the mucous membranes, particularly in the oropharynx. The blood count, however, differs from that usually found in agranulocytic angina, as well as from other forms of leucopenia, in that there is in addition to the profound leucopenia a relative polynucleosis of the nonsegmented neutrophils. In all cases of agranulocytic angina reported in the literature, the leucopenia has been the result of a marked diminution or absence of the polymorphonuclear neutrophils, which in fatal cases usually have disappeared completely from the circulating blood as well as from the bone marrow (Schultz¹).

Ulcerations of the mucous membranes and an extreme leucopenia may occasionally be present in other conditions simulating agranulocytic angina, notably in leucopenic leukemia, aplastic anemia, benzol and arsphenamine poisoning, and poisoning from radium or x-rays. In these conditions, however, marked alterations are also found in the hemoglobin, red blood cells or platelets, and at times immature myeloid cells are present in the peripheral blood. Ulcerations in the oropharynx, and rarely leucopenia may likewise occur at the onset of infectious mononucleosis (McKinlay and Downey²).

The three cases to be reported in this paper, although clinically resembling agranulocytic angina, showed a relative polynucleosis (nonsegmented neutrophils), associated with a profound leucopenia, with a more or less parallel reduction in the lymphocytes and monocytes. The red blood cells and platelets remained unaffected.

Detailed reports of these cases follow:

CASE 1.—I. K., female, aged fifty-two, married; admitted to private pavilion of Mount Sinai Hospital (Service of Dr. A. A. Epstein), December 19, 1923.

Previous History.—Following an operation for fibroids eleven years previously, the patient developed persistent and irregular pain in the epigastrium, which became severe while eating, but subsided somewhat about two hours after the ingestion of food. From time to time there was considerable nausea, but no vomiting. The appetite was poor; the diet consisted chiefly of crackers and tea. The bowels were constipated. The patient entered the hospital for an exploratory laparotomy.

Examination on Admission.—The patient was well developed. The mouth, throat, heart, and lungs were normal. The liver and spleen were not palpable. There was slight tenderness in the epigastrium. The urine showed a faint trace of albumin and occasional hyaline casts.

*From the Wards and Laboratories of the Mount Sinai Hospital.
Received for publication, January 6, 1923.

The blood examination showed a slight leucopenia:

Hemoglobin	85 per cent
Red blood cells	4,000,000
White blood cells	4,500
Polymorphonuclear neutrophiles	55 per cent
Lymphocytes	45 per cent

Course and Treatment.—An exploratory laparotomy was performed by Dr. A. A. Berg on Dec. 26, 1928. A diverticulum on the posterior wall of the duodenum was found. Otherwise, no other abnormality was noted. Following the operation, the temperature rose to 101.4° F. and remained around that level for two weeks.

About one week after the operation (Jan. 2, 1929), patient complained of a sore tongue and considerable pain on swallowing. Extensive ulcerations were found not only on the tongue, but also on the tonsils, uvula, pillars of the fauces, and on the hard and soft palates.

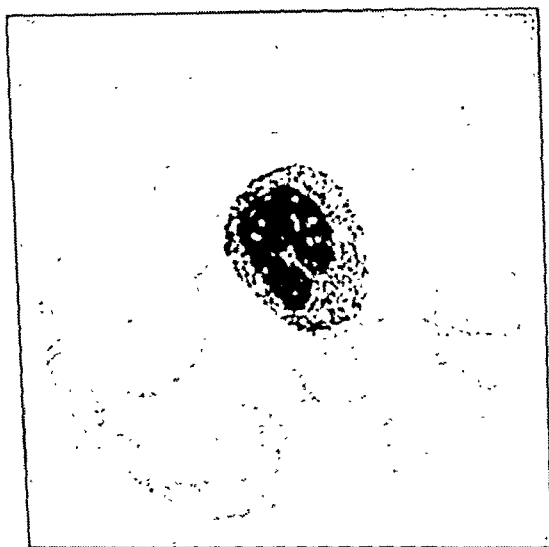


Fig. 1.—Nonsegmented neutrophiles in the peripheral blood showing "toxic" granules in the cytoplasm.

These were covered with yellowish membranes, easily removed without bleeding. Cultures of the ulcerations were negative for diphtheria bacilli and smears showed numerous streptococci and bacilli; and few spirilli. The patient became progressively weaker, as the condition of the mouth and throat did not respond to local treatment. Transfusion (500 c.c.) was followed by temporary improvement.

Three days later (Jan. 5, 1929), as the prostration, fever (104° F.), and oropharyngeal necroses became pronounced, the clinical symptoms suggested agranulocytic angina. The blood picture was compatible with this diagnosis, with the exception of the differential count. There was a relative increase in the percentage of the nonsegmented neutrophiles (the staff and young cells of the Schilling hemogram), associated with a profound leucopenia (Table I).

The neutrophiles, both segmented and nonsegmented, presented severe toxic changes (Fig. 1) in the cytoplasm. These consisted of the appearances of coarse granules and vacuolization. All the neutrophiles in this case showed such toxic changes as well as anisocytosis and poikilocytosis.

These abnormal blood changes persisted (Table I).

TABLE I

CASE 1

	JAN. 5, 1929	JAN. 6, 1929	JAN. 7, 1929 BEFORE R'ADIO- THERAPY	JAN. 7, 1929 6 HOURS AFTER R'ADIOTHERAPY
Hemoglobin	70%	74%	70%	
Red blood cells	3,400,000	3,800,000	4,000,000	
White blood cells	2,200	900	1,000	1,600
Platelets	450,000	430,000	240,000	
Polymorphonuclear nonseg- mented cells	52%	65%	44%	57%
Polymorphonuclear segmented cells	5%	2%	5%	8%
Lymphocytes	35%	26%	40%	29%
Monocytes	4%	4%	7%	4%
Plasma cells				1%
Macrophages			1%	
Myelocytes neutrophilic	4%	2%	3%	
Degenerative index	100%	100%	100%	100%

Comment.—The severe leucopenia associated with a high percentage of nonsegmented neutrophils in itself was an unfavorable prognostic sign. The number of neutrophils with toxic granules in the cytoplasm (Fig. 1) was very high (100 per cent). Such cytoplasmic toxic degeneration has been previously noted by Gloor,³ Mommensen and others.⁴ The quantitative measure of these pathologic neutrophils is obtained by dividing their number by the total number of neutrophils. This has been previously designated by us⁵ as the *degenerative index*. The persistence of a degenerative index of 100 usually indicates an unfavorable prognosis. The rapid increase of the degenerative index is usually associated with an unfavorable outcome. The index, therefore, may be regarded as a valuable aid in prognosis.

CASE 2.—R. C., female, aged sixty-two, admitted to Mount Sinai Hospital (Medical service of Dr. George Baehr), July 8, 1930, as a case of agranulocytic angina.

Previous History.—For six years preceding, the patient's complaints were chiefly cardiac, dyspnea, palpitation, and precordial pains.

Present Illness.—Six days previous to admission, there was generalized abdominal pain and diarrhea, followed by fever, progressive weakness, angina, and increasing dysphagia. Four days previously, swelling of the neck and parotid regions developed.

TABLE II

CASE 2

	JULY 8, 1930	JULY 9, 1930	JULY 10, 1930	JULY 11, 1930	JULY 12, 1930	JULY 13, 1930
White blood cells	1,800	2,200	11,600	10,000	9,600	19,900
Polymorphonuclear non- segmented cells	39%	44%	54%	50%	50%	60%
Polymorphonuclear seg- mented cells	10%	13%	11%	30%	39%	12%
Lymphocytes	33%	30%	21%	6%	9%	16%
Monocytes	13%	1%	12%	10%	2%	11%
Myelocytes	5%	6%	2%	4%		1%
Degenerative index	100%	100%	100%	100%	100%	100%

Examination on Admission.—The patient was obese, flushed, toxic in appearance, and somewhat stuporous. Several lesions resembling pustules were present on the skin. The mucous membrane of the mouth was dry, glazed and reddened; a large necrotic black slough was adherent to the palate and extended to the pillars of the fauces and the posterior pharyngeal wall. Tender swellings were found in both submaxillary regions and over the

left parotid glands. There was slight rigidity of the neck. Heart sounds were irregular and of poor quality. Breathing was stertorous.

Course and Treatment.—The clinical course was suggestive of agranulocytic angina. The blood picture, however, showed a white cell count of 1,300 with 49 per cent polymorphonuclear neutrophils, and 39 per cent nonsegmented cells (Table II); the hemoglobin, red blood cells, and platelets were normal. Blood cultures, taken on two occasions, were negative. All of the neutrophils contained toxic granules (degenerative index 100).

Signs of myocardial failure developed and on two occasions pulmonary edema, which subsided with medication (atropine and adrenalin). There was a transitory improvement for a few days after a blood transfusion (500 c.c.). The ulcerations and leucopenia both subsided. The white cells increased rapidly (two days) to 11,600, with 65 per cent polymorphonuclear neutrophils when the patient developed a right suppurative parotitis. Following this, the condition became considerably worse with signs of pneumonia and a rise in temperature to 105.8° F. and death ensued.



Fig. 2.—Bone marrow of Case 2. Note the hyperplastic bone marrow with many mature and immature myeloid cells

Comment.—In this case the rise of the white blood cells from 1,800 to 10,000 after two days in the hospital would ordinarily be considered a favorable sign. In following the blood picture daily (Table II), two signs were significant: (1) the nonsegmented neutrophils steadily rose from 39 per cent to 60 per cent; and (2) the degenerative index persisted at 100 per cent throughout the course of the disease. Although some of the hematologic findings were suggestive of apparent recovery, the severe complications (parotitis and pneumonia) led to a fatal outcome as was prognosticated by other blood changes, i.e., the high degenerative index, and the progressive increase of the nonsegmented neutrophils.

CASE 3.—J. J., male, aged thirty-five, physician, admitted to Mount Sinai Hospital (Surgical service of Dr. Neuhoof and Otolaryngological Service of Dr. Yankauer), on Nov. 5, 1930.

Previous History.—Negative.

Present Illness.—Three days previous to admission (Nov. 2, 1930) patient had been admitted to Willard Parker Hospital, with a suspected laryngeal diphtheria following an attack of respiratory difficulty accompanied by stridor and pain in the chest. Had also complained of dysphagia and hoarseness, and an acute pharyngitis and laryngitis, and a membranous pharyngeal ulceration extending to the larynx were found. Temperature was

102° F., and later 104° F. The throat culture showed *Streptococcus hemolyticus*. The blood picture was apparently normal as the white blood cells were 6,500 with 63 per cent polymorphonuclear neutrophils. Under observation, the patient's condition became worse, with more marked laryngeal involvement. Within a few days the white blood cells diminished to 2,500 without any change in the percentage of polymorphonuclear neutrophils. He received two blood transfusions.

On admission to the Mount Sinai Hospital, the Wassermann reaction was negative. The blood examination showed a profound leucopenia with a marked increase of the non-segmented neutrophils and was as follows:

Hemoglobin	85 per cent
Red blood cells	4,800,000
White blood cells	3,500
Polymorphonuclear nonsegmented cells	65 per cent
Polymorphonuclear segmented cells	10 per cent
Lymphocytes	25 per cent
Degenerative index	88 per cent

Course and Treatment.—The stridor became more marked and the patient experienced a great deal of difficulty in breathing.

A tracheotomy was performed for suspected perilaryngeal phlegmon. The tissue was found to be edematous but no pus was present. Following the operation the temperature gradually returned to normal and the stridor appreciably decreased. Cultures from the region of the laryngeal slough revealed numerous colonies of *Streptococcus hemolyticus*. Sections made from the necrotic tissue removed from the larynx showed necrotic purulent exudate with numerous bacterial colonies. The necrotic lesions in the pharynx healed rapidly. Progress following the tracheotomy was rather slow. The condition of the larynx was considered a necrotizing process as a result of a severe streptococcic infection. The entire right side of the larynx down to the true vocal cords sloughed away. For a time this caused marked discomfort as the loss of structure resulted in aspiration of fluids on swallowing. For a few weeks, nasal feeding was necessary. The patient was also given several transfusions and frequent intravenous glucose infusions. Save for a slight pneumonic infiltration noted only on x-ray examination (Nov. 8, 1930), no complications developed. The temperature became normal about November 10.

After the fourth week in the hospital, the slough in the larynx entirely separated and healthy granulations could be seen. On examination, the distortion and the granulations had a peculiar appearance resembling a neoplastic condition. The patient's voice and general condition improved. When the laryngeal condition was almost healed, about the middle of December, a permanent tracheotomy tube with a safety valve was inserted. Since discharge from the hospital (on Dec. 12, 1930), the patient is apparently well save for the granulomatous condition in the larynx.*

The blood picture (Table II) during the course of the disease is of singular interest. The white blood cells increased to normal by Nov. 13, 1930; however, the nonsegmented cells remained increased during the entire period of observation at the hospital. The degenerative index, which gradually increased the first few days at the hospital, returned to normal about November 24.

DISCUSSION

Three cases of severe infection associated with necrotic lesions of the mucous membranes and an unusual type of leucopenia have been described.

Clinically these cases resembled agranulocytosis. The onset of hypoleucocytic angina was apparently gradual and insidious, about six days elapsing before it fully developed. The first symptom noted was soreness of the throat or tongue, associated with dysphagia. Hoarseness and marked stridor were present

*This patient died on Nov. 17, 1933, after several attacks of true agranulocytosis, which began in May, 1933.

in one case. Fever was usually slight at the onset, but increased as the disease progressed, reaching 104° to 105° F. At the height of the disease, the patients were prostrated, and appeared flushed and toxic. The tongue, tonsils, pharynx, larynx, or all of them showed extensive sloughing. Large, irregular, sharply delineated necrotic areas were present. These were covered with a yellowish or blackish membrane. Throat cultures were positive for hemolytic streptococci. No diphtheria bacilli were found. In one instance, a section of necrotic slough showed a purulent exudate with numerous bacterial colonies. In two cases, the blood cultures were negative; no blood culture was made in the third.

At the height of the disease, the blood picture showed a profound leucopenia with several unique features. Although there was a marked depression in the total white cell count, the differential count retained the usual relations. The presence of a high percentage of nonsegmented polymorphonuclear neutrophils showing degenerative changes in the cytoplasm (Fig. 1) confirmed the impression that a severe infection was present. The hemoglobin, red cell count and platelets were unaffected. In one case the bone marrow was obtained and was found to be hyperplastic with an abundance of mature and immature myeloid cells (Fig. 2). This is in striking contrast to true agranulocytic angina, in which at the height of the disease, there is ordinarily a complete disappearance of the granulocytes from the blood stream, as well as from the bone marrow.

CONCLUSION

A study of the blood picture in the three cases described in this article revealed an unusual form of leucopenia in a condition termed by the authors "hypoleucocytic angina" to describe a clinical syndrome similar to that of agranulocytic angina, but which differs hematologically from the latter as well as from other forms of leucopenia, in that there is a relative polynucleosis of the nonsegmented neutrophils.

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THE GRAM PROPERTY OF THE ACID-FAST FORM OF THE TUBERCLE BACILLUS*

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IT IS stated in standard textbooks of bacteriology¹ that the tubercle bacillus is gram-positive. It seems that this statement has never been seriously challenged. This property of the tubercle bacillus is of more than mere theoretical interest, it is of definite diagnostic importance due to the claim that certain bodies, called Much "granules," are nonacid-fast gram-positive forms of a hitherto unknown tuberculosis virus which are often alleged to be found instead of the typical acid-fast tubercle bacilli within cold abscesses and in some sputa. The questions raised in this and in a succeeding paper are: (1) Does the tubercle bacillus possess the Gram staining property? (2) Is the Much "granule" stain a modified Gram stain?

DOES THE TUBERCLE BACILLUS POSSESS THE GRAM STAINING PROPERTY?

The purpose of this paper is to demonstrate that the tubercle bacillus in its pathogenic acid-fast phase does not possess the genuine Gram property; that although it is credited with being gram-positive even in the most authoritative treatises,¹ the tubercle bacillus, in its acid-fast stage, is by nature of its physicochemical constitution an organism to which the Gram staining method cannot properly be applied. It is here advisedly stated that, in its pathogenic stage the tubercle bacillus is acid-fast, or, conversely, that the nonacid-fast forms of the organism have not yet been shown to produce typical tuberculosis in the experimental animal. It is true that Much claims by means of his so-called modified Gram stain to have discovered an atypical nonacid-fast gram-positive form of the tuberculosis virus. In a succeeding paper I have taken issue with part of this contention. Sweeny² by means of the single isolation method of Janse-Peterfi in modified form, produced a pathologic condition by the injection of a single nonacid-fast coccoid mutation form of the tubercle bacillus. The disease so produced is not, however, a typical tuberculosis.

When aniline, as employed in the standard Gram stain, is used in combination with a basic stain such as gentian violet or basic fuchsin in staining the acid-fast form of the tubercle bacillus, it renders these dyes acid-fast similar to the action of phenol. This action of aniline is not surprising since it is phenylamine, a benzene ring compound to which class of chemical compounds belongs also phenol. Even before the Ziehl acid-fast method of staining the tubercle bacillus was devised, Ehrlich³ employed a solution of gentian violet in aniline water for demonstrating the tubercle bacillus. He found that this stain resisted decolorization with 33 $\frac{1}{3}$ per cent nitric acid when

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applied to this organism. Hence, instead of the phenol of the carbol-fuchsin, the aniline served with similar efficiency in bringing about retention of the original dye; the aniline dyes were acid-fast similar to the carbol-dyes and likewise alcohol-fast, since Ehrlich's method employed 60 per cent alcohol as decolorizer after application of the acid.

Benians⁴ states that the tubercle bacillus does not require iodine to enable it to withstand alcohol and acid-alcohol decolorizers. He fails to cite experimental work. The same writer says, likewise, that in presence of the acid-fast property it is impossible to fully demonstrate alcohol-fastness, i.e. in the face of acid-fastness, the Gram property cannot be fully exhibited.

EXPERIMENTAL

General Technic.—Untreated tubercle bacilli from a pure culture were applied to slides in a drop of distilled water, and dried in a paraffin oven at 55° C. for a few minutes. No fixation was applied to cause the organisms to adhere so as to prevent the introduction of artefacts.

I. COLD STAINING OF THE TUBERCLE BACILLUS WITH THE GRAM STAIN

Experiment 1.—Tubercle bacilli were stained according to the standard Gram stain but no counterstain was used. The bacilli were stained cold with aniline gentian violet (Stirling's) for one minute, Gram's iodine solution one minute, and decolorized with alcohol until color ceased to come off; no counterstain was applied.

Result: The tubercle bacilli were stained an intense deep purple color.

Experiment 2.—Tubercle bacilli were stained with formalin gentian violet, which is occasionally used in the Gram stain, cold for one minute, Gram's iodine solution one minute, decolorization with alcohol, one slide with and one without counterstain.

Results: Neither slide showed any stained tubercle bacilli.

Experiment 3.—Tubercle bacilli were stained with cold aniline fuchsin, the remainder of the procedure as in (2) above.

Result: The organisms were not stained.

Experiment 4.—Tubercle bacilli were stained with cold crystal violet as carried out by Ruhland,⁵ the remainder of the procedure as in (2) above.

Result: No appreciable staining of bacilli.

Experiment 5.—Purpose: To ascertain the effect of carbol-fuchsin on the tubercle bacillus when applied cold. A preparation of a slide of tubercle bacilli was stained cold with carbol-fuchsin for one minute, not treated with iodine solution nor decolorized with alcohol.

Result: The bacilli were stained faintly or not at all.

Comments on Experiments 1 to 5: Carbol-fuchsin, aniline fuchsin, and crystal violet do not stain the tubercle bacilli when applied cold (for one minute); aniline gentian violet, however, stains the tubercle bacilli intensely when applied cold for one minute, nor does it decolorize with alcohol. Whereas, phenol in combination with fuchsin will stain the tubercle bacilli when applied hot, it fails to stain them when applied cold; aniline, on the other hand, in combination with fuchsin fails to stain the tubercle bacillus when applied cold but when combined with gentian violet does so readily. Formalin gentian violet and crystal violet dyes that are frequently used in Gram staining fail to stain the tubercle bacillus when applied cold.

It will thus be seen that aniline fortifies only gentian violet in cold staining.

II. EFFECT OF IODINE ON THE TUBERCLE BACILLUS

a. *With Alcohol as Decolorizer.*—

Experiment 6.—"Gram" stain without iodine solution. Tubercle bacilli were stained with aniline gentian violet as in Experiment 1 but using no iodine; decolorization with alcohol.

Result: The organisms were stained as intensely as when iodine was used.

Comment on Experiment 6: Iodine is not necessary to "mordant" the aniline gentian violet as is the case with nonacid-fast organisms in order to enable the tubercle bacilli to retain this stain. Hence, one of the essential stages in the classical Gram stain—the "mordanting" with iodine, has thus been shown to be unnecessary when applied to the tubercle bacillus to enable the latter to retain the gentian violet when alcohol is employed as the decolorizing agent.

b. *With Acid-Alcohol as Decolorizer.*—

Experiment 7.—Tubercle bacilli were stained with cold aniline gentian violet for one minute, Gram's iodine solution one minute, acid alcohol (3 per cent HCl in 70 per cent alcohol) until color ceases to come off. This acid alcohol is the same as that employed in the Ziehl-Neelsen stain.

Results: Tubercle bacilli were intensely stained a dark blue color; they were not decolorized by the acid alcohol; in other words, they were acid-fast to the Gram stain.

Experiment 8.—Same as Experiment 7, but without the Gram's iodine solution.

Results: The tubercle bacilli were stained as intensely as in Experiment 6.

Experiment 9.—Living cultures of recently isolated gram-positive pathogenic staphylococci and streptococci were stained according to Gram and decolorized with acid alcohol as employed in the Ziehl-Neelsen stain. They were then counterstained with Bismarck brown.

Results: These gram-positive organisms were completely decolorized by acid alcohol and took the counterstain.

Comment on Experiments 7, 8, and 9: The tubercle bacilli will retain the aniline gentian violet as well when the organisms are not "mordanted" with Gram's iodine solution as when they are so treated, not only after alcohol but also after acid alcohol is applied as decolorizer. This is not the case with pyogenic gram-positive organisms such as staphylococci and streptococci. When these are exposed to acid alcohol even after application of iodine solution they will lose their gentian violet, which is to be expected since they are not acid-fast but only alcohol-fast (gram-positive); the tubercle bacillus, however, is alcohol and acid-fast. This constitutes, consequently, a basic deviation from the Gram stain in the case of the tubercle bacillus, so vital as to force the conclusion that the tubercle bacillus does not possess genuine Gram property since, first, it does not require iodine to remain alcohol-fast, and second, the bacillus stained with aniline gentian violet is acid-fast.

STANDARD GRAM STAIN APPLIED TO CHEMICALLY TREATED TUBERCLE BACILLI

Living tubercle bacilli from cultures were deprived of their acid-fastness by strong acid and alkali. This often is rather difficult to accomplish completely in all of the tubercle bacilli treated, if the morphology of the organisms is to be retained. The purpose of this mode of experimentation was to ascertain the reason for the atypical Gram property of the tubercle bacillus, in

which portion of the tubercle bacillus it is vested, and to determine the Gram property of the tubercle bacillus which has been deprived of its acid-fast property.

Tubercle Bacilli Treated With Sodium Hydroxide.—As Long⁶ has pointed out, the acid-fastness of the tubercle bacillus is highly resistant to this alkali in rather strong solutions. In the following experiments living tubercle bacilli were dried and pulverized by passing through a fine mesh copper sieve (1,936 per sq. in.) and exposed in centrifuge tubes to a 25 per cent solution of sodium hydroxide for varying periods of time. The hydroxide was then carefully decanted so that the bacilli adhered to the wall of the tube, and the organisms washed and centrifuged several times until the washings were neutral to litmus. They were then applied without fixative to slides, placed in the paraffin oven at 55° C. for a few minutes until dry and then stained.

Experiment 10.—Exposure time to 25 per cent sodium hydroxide: five minutes.

Result: Tubercle bacilli well stained with carbofuchsin. The granules seemed somewhat swollen. The intergranular portion of the bacilli generally is not stained with carbofuchsin, it often remains colorless as is frequently the case in untreated bacilli.

Experiment 11.—Exposure time same as Experiment 10. The organisms were Gram stained after application of hydroxide. This shows the granules stained very intensely purple. The intergranular material in many bacilli stains brown with the counterstain (Vesuvín). Hence, we have the apparent anomaly of an organism staining "gram"-positive and "gram"-negative in the same bacillary body.

Experiment 12.—Exposure time: one and one-half hours.

Results: Ziehl-Neelsen stain showed a number of nonacid-fast tubercle bacilli which stained faintly blue with the methylene blue. Those that stained acid-fast were colored a very faint red.

Experiment 13.—Exposure time: one and one-half hours. Gram stain applied.

Results: These tubercle bacilli are stained more intensely with aniline gentian violet than with carbofuchsin in Experiment 10. Some take the counterstain, i.e. they are colored brown by the vesuvín. These tubercle bacilli that have been rendered gram-negative, stain uniformly throughout with no granules whatever, and the bacillary body is intact.

Experiment 14.—Exposure time: four days. Practically all the tubercle bacilli have been disintegrated. However, even after this period of time a rare bacillus staining acid-fast and "gram"-positive is seen.

Experiment 15.—Tubercle bacilli not treated with sodium hydroxide were exposed to Bismarek brown, heating the stain for one minute. No stained tubercle bacilli were to be seen. Some of the extrabacillary amorphous material only stains brown. This effect of Bismarek brown on the tubercle bacillus is in keeping with the work of Hope Sherman.⁷

Comments on Experiments 10 to 15: After being exposed to sodium hydroxide the tubercle bacillus can be made to manifest an apparent anomaly of being simultaneously "gram"-positive and "gram"-negative in the same bacillus. This is explainable by assuming that the sodium hydroxide brings about changes in the intergranular portion of the bacillus facilitating penetration of the Gram counterstain, Bismarek brown, which by itself (Experiment 15), will not adequately stain the untreated tubercle bacillus. This intergranular portion of the untreated tubercle bacillus is often quite resistant to strong dyes as is evidenced by unstained areas between the granules of many of the organisms after the Ziehl-Neelsen stain. This double staining

after treatment with sodium hydroxide can be explained on the basis of Experiments 6, 7, 8, and 9, above. The aniline gentian violet of the Gram stain, as has been shown in these experiments acts as an acid-fast stain for the acid-fast portion, especially the granules of the tubercle bacillus in these treated organisms, and the investment of the nonstaining intergranular portion having been removed or altered by the hydroxide is rendered permeable to the Bismarck brown and shows genuine gram-negative property. As has been seen in Experiment 15, Bismarck brown does not appreciably stain the untreated tubercle bacillus.

Tubercle Bacilli Treated With Sulphuric Acid.—The purpose of this work was to render the tubercle bacillus nonacid-fast with sulphuric acid in a rapid and certain manner and then to study the Gram property of the nonacid-fast organism.

Experiment 16.—Dried living tubercle bacilli that were passed through a fine sieve were exposed to the action of 2.5, 7.5, 10, 15, 20, 50, and 75 per cent sulphuric acid solutions.

Results: The tubercle bacilli in the center of small clumps often remained acid-fast even in the higher percentages, after five minutes' exposure. Hence concentrated sulphuric acid was employed to deprive the organisms of their acid-fastness completely and as quickly as possible without altering their morphology.

Experiment 17.—Concentrated sulphuric acid (sp. gr. 1.84) was applied to pulverized intact tubercle bacilli. The latter floated on the surface and could, after exposure time had elapsed, be skimmed off with a coiled platinum loop and washed with distilled water into a centrifuge tube. The tubercle bacilli were then centrifuged, and repeatedly washed until the wash water was neutral to litmus. The organisms were then applied to slides, dried at room temperature, and stained. When the tubercle bacilli were exposed much longer than five minutes to concentrated sulphuric acid, they generally disintegrated and showed mostly amorphous debris which stained gram-negative. One-minute exposure showed that some of the tubercle bacilli were losing their acid-fast material which latter appeared as round or oval droplets in the optical fields. Often these were still attached to the bacillus which stained blue (nonacid-fast), and the former bright red (acid-fast) (see Plate I).

Results: Thus the acid-fast material was "extracted" by the sulphuric acid from the tubercle bacilli and the bacillus was left morphologically intact, staining gram-negative, if treatment was not too prolonged so as to produce disintegration of the bacillary bodies.

Five minutes' exposure at room temperature brought about a complete abolition of acid-fastness. The bacilli were stained uniformly throughout the bacillary body a bright blue by the contrast stain. With the Gram method of staining the same bacilli stained a brilliant brown with the resubin, the dye being evenly distributed throughout the bacillus and the latter free from all beading. Hence they had been rendered gram-negative by removal of the acid-fast material. The morphology and staining of these tubercle bacilli deprived of their acid-fastness were identical with the staining of nonacid-fast gram-negative young bacilli found in a culture obtained directly from sputum grown on potato medium.

Comment on Experiments 16 and 17: From these experiments it will be seen that after the acid-fast materials in the tubercle bacillus have been removed or destroyed chemically there remains a bacillus "underneath" that is gram-negative. In other words, deprived of its acid-fast material which has no genuine Gram property, the "basic portion" of the tubercle bacillus is gram-negative. This Gram counterstain colors the entire tubercle bacillus uniformly throughout with the Bismarck brown counterstain, both what was the granular and intergranular portion of the organism. These bacilli stained

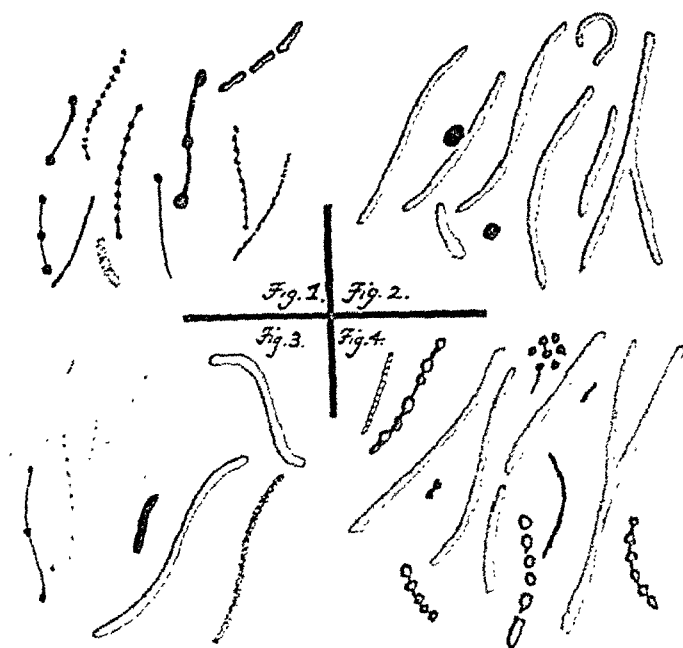


Fig. 1.—Acid-fast beaded tubercle bacilli which were treated with sulphuric acid, with the results seen in Fig. 2. Note how slender they are in comparison with nonacid-fast forms in Figs. 2 and 3.

Fig. 2.—Ziehl-Neelsen stain of tubercle bacilli after treatment with concentrated sulphuric acid for one minute. Bacilli have taken the counterstain: they are nonacid-fast. Acid-fast bacilli are still clinging to a tubercle bacillus, are shown. These latter bacilli were gram-negative. The beading has disappeared. Tubercle bacilli stain uniformly and their morphology is intact. They resemble the nonacid-fast bacilli seen in Fig. 3.

Fig. 3.—Culture stained with Ziehl-Neelsen stain, showing acid-fast, some nonacid-fast and partially nonacid-fast forms. Note similarity of the morphology of the "young" nonacid-fast bacilli to those deprived of their acid-fastness by sulphuric acid shown in Fig. 2.

Fig. 4.—Gram stain (Bismarck brown counterstain) of a culture of tubercle bacilli containing some nonacid-fast tubercle bacilli shown in Fig. 2. The bacilli are heavy rods and stain evenly without beading.

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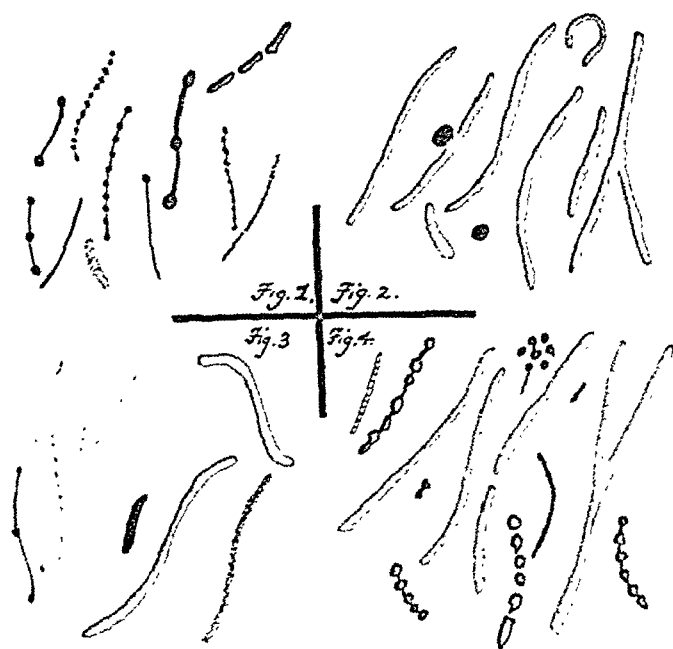


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with the counterstain possess none of the aberrant morphology of the untreated acid-fast tubercle bacilli, but are uniform rods of varying lengths, with rounded ends. Moreover, the tubercle bacillus may be violently deprived of its acid-fastness and of its so-called gram-positivity and yet retain its morphology, that is, not to be disintegrated. Benians⁴ found that mechanical disruption of the body of the tubercle bacillus destroyed both its acid-fastness and its "gram-positivity" and rendered the bacillary débris gram-negative. However, it will be seen from the above work that the tubercle bacillus may be deprived of its acid-fast property completely without causing disintegration of the bacillary body. The capsule of the bacillus, if it has one, following treatment with sulphuric acid seems to remain intact as the bacillus retains a definite bacillary morphology after such exposure. The bacillus so deprived of its acid-fast material appears wider than the average tubercle bacillus, has rounded ends, and stains uniformly throughout without granulations or unstained areas. The impression one receives is that the tubercle bacillus consists primarily of a gram-negative bacillus (young organism) of rather uniform staining property which in the course of its metabolism becomes encrusted externally to its investment ("capsule") or within the latter with acid-fast, lipoidal material which is frequently unevenly distributed in the form of granules. Simultaneously with this process the "capsule" becomes impervious to all dyes as is evidenced by the unstained portions of the bacillus between the granules. The presumption is that the hydroxide dissolves off this investment.

STAINING OF THE ETHER EXTRACT OF THE TUBERCLE BACILLUS

Experiment 18.—Tubercle bacilli were extracted with ether for ten months. This ether extract was placed on slides and the ether allowed to evaporate. The slides were then stained according to Ziehl-Neelsen and Gram methods.

Result: The former showed only slightly acid-fast staining property; the latter stained a deep purple.

Comment: It would appear that the lipoidal extract of the tubercle bacillus is more "gram"-positive than acid-fast, due probably to the greater resistance of aniline gentian violet than carbolfuchsin to acid alcohol and also to the greater visibility of the former. The ether extract is, like the unextracted lipoid in the tubercle bacillus, both alcohol- and acid-fast and hence has no genuine gram-positive property since the latter demands only a resistance to alcohol (alcohol-fastness).

DISCUSSION

In order to clarify the discussion it might be well to define what is meant by the Gram and by the acid-fast property. For an organism to possess the Gram property it must either lose or retain the original stain which has been "mordanted" with the special Gram's iodine solution, when alcohol or acetone are applied as decolorizers. In the former case the organism is "gram-negative," in the latter "gram-positive." An organism is acid-fast if the dye with which it has been stained fails to be removed when treated with dilute mineral acids. Incorporated with this acid-fast staining solution is some chemical

such as phenol to aid penetration. In addition it may be necessary to apply heat to bring about penetration into the microorganism. Alcohol is not an essential decolorizing ingredient in the acid-fast staining method, it being employed on the assumption that the tubercle bacillus is more resistant to it than are certain other acid-fast organisms such as the *B. lepra* and the smegma. Stitt,⁸ however, states that he has found smegma bacilli often to be quite resistant to alcohol.

In Experiment 6 it was found that the tubercle bacillus requires no iodine to enable it to retain the original Gram stain in the face of alcohol decolorization. In Experiment 8 it was seen that the tubercle bacillus retains the Gram dye (aniline gentian violet) not only when alcohol, but also when acid alcohol is applied. Hence, it must be concluded that the tubercle bacillus does not possess the typical Gram property, in fact the Gram dye (aniline gentian violet) actually proves to be acid-fast for this organism, since it is not decolorized by 3 per cent HCl in 70 per cent alcohol. The other dyes sometimes employed for Gram staining such as crystal violet and formalin gentian violet do not even adequately stain the organism. As seen in Experiments 1 and 2 the only one of these Gram dyes that will penetrate the tubercle bacillus when applied cold is aniline gentian violet. Not even carbolfuchsin will do so. In short, the acid-fast property of the tubercle bacillus precludes the possibility of its possessing the real Gram property.

From these staining experiments one is led to believe that both the acid-fast property and the "gram-positivity" of the tubercle bacillus are attributable to the same substance or substances in this organism. In order to ascertain this, tubercle bacilli were exposed (Experiments 10 to 17) to a 25 per cent solution of sodium hydroxide and to concentrated sulphuric acid with the following results: (1) Complete removal of the acid-fast property and at the same time of the "gram-positivity." This would seem to indicate that both properties depend upon the same substance or substances. This appears to be confirmed further by Experiment 18 in which the ether extract retains both the carbolfuchsin and the aniline gentian violet of the Gram staining method. (2) When tubercle bacilli are exposed for a very short time to strong acid or alkali, they may still remain acid-fast, but when stained according to Gram, part of the individual bacillus may stain "gram-positive" in the granular portion of the organism, and part of the bacillus stains gram-negative in the areas between the granules. This merely confirms the previous finding, under (1), of the identity of the acid-fast and of the "gram-positive" material. A change in the "capsule" in the intergranular area seems to have been produced by acid and by alkali, permitting the passage of the Gram counterstain where ordinarily the Bismarck brown does not penetrate, it being the most resistant portion of the tubercle bacillus to stain. The granules on the other hand are the acid-fast areas of the organism. (3) When tubercle bacilli were treated with concentrated sulphuric acid for a short time, droplets appeared outside of the organisms which retained both the carbolfuchsin and the aniline gentian violet in the face of acid-alcohol decolorization, they were acid-fast, still more confirming the opinion that both acid-

fast and "Gram" properties depend upon the same substance when applied to the tubercle bacillus. (4) Tubercle bacilli deprived of their acid-fast material by means of alkali or acid, stain gram-negative in the true sense, that is, they are decolorized by alcohol after previous application of Gram's iodine solution.

In studying the morphology of tubercle bacilli that have been deprived of their acid-fastness a striking resemblance was observed between them and young nonacid-fast tubercle bacilli in that: (a) their morphology is that of uniform rods with rounded ends and of varying lengths, but of remarkably uniform diameter, which latter is much greater than that of the average tubercle bacillus (see Plate I); (b) uniform distribution of stain throughout the bacillary body, with entire absence of beading. The bacilli are less bizarre than is the acid-fast form of organism. One is consequently led to conclude that when the tubercle bacillus is stripped of its unusual property that sets it apart from most other pathogenic organisms, namely, its acid-fastness, it is gram-negative. When the young nonacid-fast tubercle bacillus assumes acid-fastness, one may picture it as becoming impregnated, or perhaps encrusted with a waxy lipoidal covering which does not always distribute itself uniformly but often in beaded fashion and is bound to the capsule in a physico-chemical union. From the manner in which the acid-fast material is removed by sulphuric acid and by sodium hydroxide, as seen in this work, leaving a morphologically intact bacillus underneath, it would seem likely that this material merely forms an investment over or within the capsule, but not within the bacillary protoplasm, otherwise one would expect a disturbance in the morphology of the bacillus consequent upon its removal.

CONCLUSIONS

1. The only Gram dye that adequately stains the tubercle bacillus when applied cold is aniline gentian violet. This dye has greater penetrability than the classical carbolfuchsin of the acid-fast technic which has to be applied hot.

2. It has been found in the above work that when aniline gentian violet used in the classical Gram stain has been applied, the tubercle bacillus will retain this dye not only after application of alcohol but also after decolorization with acid alcohol (3 per cent HCl). Consequently this Gram dye proves to be acid-fast for the tubercle bacillus.

Thus it will be seen that the acid-fast form of the tubercle bacillus *possesses no true gram-positive property* because the acid-fast property renders this impossible since none of the other Gram dyes penetrates the organism under like circumstances (applied cold).

3. When the tubercle bacillus is completely deprived of its acid-fast property either by strong acid or alkali, it becomes gram-negative, similar to young cultures which likewise often contain nonacid-fast tubercle bacilli that stain gram-negative. On the other hand, so long as the tubercle bacillus retains the "Gram" stain, it will also invariably be found to be acid-fast, if not with carbolfuchsin, at least with carbol or aniline gentian violet.

4. When the tubercle bacillus is deprived by acid or alkali of its acid-fast property, it loses its beading and stains uniformly, becomes gram-negative, and resembles the young nonacid-fast form of the organism.

5. The suggestion is herewith offered that in the future, writers of books and articles on tuberculosis when describing the staining properties of the tubercle bacillus state that in its acid-fast pathogenic form it does not possess true Gram property; that the Gram technic, as a differential stain, cannot be applied to this organism because of the presence within it of the acid-fast material.

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VARIATIONS OF THE SOLUBILITY OF THE CERVICAL MUCUS IN RELATION TO THE MENSTRUAL CYCLE*

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IN A VERY painstaking series of experiments Kurzrok¹ has investigated the lytic action of semen on cervical mucus. He found that normal semen digests normal cervical mucus and suggests that either changes in the lytic action of the semen or in the character of the cervical mucus or both may produce sterility. Mucopurulent cervical secretion for instance Kurzrok has always found to be nondigestible by semen.

In the course of examining sterile couples I have repeated some of Kurzrok's experiments using mainly the gross method of immersing the cervical mucus in the semen. I consider this gross method less subject to misinterpretation than the microscopic penetration method as far as the solution or digestion of the cervical mucus is concerned.

The results of my experiments were not quite as expected and appeared at first to be rather fortuitous. Thus using a normal semen from a clinically fertile man on four different, apparently normal, mucus shreds from the cer-

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vices of four women I found varying degrees of solubility. At the same time the microscopic penetration method showed, to my eyes at least, no significant variations as far as the penetration of the cervical mucus by the spermatozoa was concerned. Again in four sterile couples where I used the husband's semen with the apparently normal cervical mucus of the wife and also on three of these the semen of the known fertile man my results were inconclusive since the semen from the normal man did not produce much dissolution of the cervical mucus from two of the four women who were apparently normal. However in three cases where the two semen specimens had been used similar results were obtained with both semen samples.

As far as infected mucus was concerned I obtained practically no digestion of it with semen by the gross method but did see penetration of the spermatozoa into the mucopus. The degree of penetration seemed to depend solely on the viscosity of the mucopus which thus constituted a mechanical barrier to the progress of the sperms. With rather fluid pus penetration of the sperms was evident and I have shown before² that pus itself does not inhibit the spermatozoa. That the gross digestion of the cervical mucus by the semen did not at times agree with the penetrability of the same cervical mucus by the sperms may easily be explained by assuming that the fibrin and pus in the specimen may form a network or a sort of skeleton enmeshing the mucus itself. So even if the mucus itself were dissolved no gross changes would be prominent in the original specimen from the cervix. This theory was verified to some extent by the microscopic appearance of the mucus shred after it had been subjected to the influence of the semen for some time. At any rate there was less free mucus in the cervical mucus specimen of this type after immersion in the semen for a while than there had been before.

The assumption of the penetrability of the cervical mucus by the spermatozoa varying directly as its viscosity agrees very well with clinical experiences since markedly infected cervices in which the canal itself is not plugged by the tenacious mucopus frequently allow of pregnancy whereas those cervices which are plugged up solidly by a thick mucopurulent shred prevent pregnancy just as long as the condition remains unrelieved, other conditions of course being normal.

Thus the penetration of the cervical mucus seemed to me to hinge more on the viscosity of the mucus or mucopus itself than upon the lytic action of the semen. At the same time no one will deny that, just as the number, the motility, the morphology, and the biometrics of the sperms may vary so also may the lytic action of the semen be variable. Thus while far from denying any of the interpretations Kurczok made in his experiments I could not from my own few cases (11 at that time) see any correlation between the solubility of the cervical mucus in the semen and fertility except so far as the viscosity of the cervical mucus when marked prevented the ascension of the sperms but this was then a purely mechanical and not a chemical process.

Some time after these experiments were made I had occasion to try out again the solubility of the cervical mucus in semen in investigating another sterile couple. I found that the cervical mucus which was apparently normal

was only slightly soluble in the semen of the husband. As a control I used the same cervical mucus with the semen of a known clinically fertile man and again obtained the same result. As a second control I tested out the same two semens on the cervical mucus of the perfectly normal wife of the fertile man and obtained a fairly marked digestion of the mucus by both semen specimens. These results now could mean only one of three things, either my technic was wrong, or there existed selective fertility inasmuch as a particular normal semen would not dissolve one normal specimen of cervical mucus whereas it would attack another such normal specimen, or there were undetected factors acting on the cervical mucus or the semen which would influence the reactions obtained.

As far as my technic was concerned I could discover no flaws in it but rechecked the findings the next day by means of the microscopic penetration method. However, this method did not offer any solution of the problem. My gross method was the same as that used by Kurzrok except that I used undiluted semen since dilution not only diluted the semen but also changed the osmotic pressure, the buffer reaction of the semen, etc. However, using the semen undiluted should if anything have made my results more accurate instead of confusing.

As far as selective fertility is concerned I have discussed this question in previous papers.³ Since all the known human races do produce fertile offspring by interbreeding, it seems illogical to assume that nature would deliberately limit procreation to certain chance combinations of a special class such as a particular type of semen combined with a particular type of cervical mucus. A similar attempt to explain some cases of apparently selective fertility has also been made in connection with the various blood groups but investigations have showed no foundation for this theory. The explanation of selective fertility is usually very much simpler (see Moench, l.c.).

There remained then only the third possibility, that of some undetected change in either the semen or the cervical mucus.

Two weeks later I was able to recheck my findings on the same patients and obtained the unexpected result that the cervical mucus of the childless woman was now more soluble in the two semen samples than before and more soluble than the mucus from the cervix of the fertile woman. This latter sample of mucus indeed was very little affected by the semen. A repetition of the tests the next day again gave the same results but a different specimen of mucus showed some solubility in the semen. This could only mean that the cervical mucus in both of these women had undergone some change. On checking up the history of the patients it was found that in the first experiment the sterile woman had been in the premenstrual period and the fertile one in the first half of the menstrual cycle whereas two weeks later the conditions were of course reversed.

In view of these results I next tried out cervical mucus from two pregnant women in the seventh and ninth month of pregnancy respectively with the semen of the same fertile man and also with the semen of another normally fertile man but obtained no dissolution of the cervical mucus whatsoever. The cervical mucus from two cases of amenorrhea was also tested but the

results were inconclusive. One specimen of mucus showed some digestion, the other none at all. However, we were not in the position to determine the amount of female sex hormone in the blood of the two patients and without this determination can draw no conclusions.

Unless then we assume that the lytic action of the semen varies without any apparent cause we must draw the conclusion that the solubility of the cervical mucus varies with different times of the menstrual cycle. This is not a difficult thing to believe. Even clinically the cervical mucus in the same woman seems at times to be more fluid than at other times. It is known that follicular fluid and also the normal secretion of the fallopian tubes and the uterine body⁴ dissolve the cervical mucus. In fact the rubbery consistency of the cervical plug during pregnancy has been explained as being due to the absence of the solvent action of the combined tubouterine secretions. That these secretions, especially the uterine one, are dependent more or less on ovarian activity is well known. Thus the concept of the varying properties of the cervical mucus is nothing new. Perhaps we have in addition a direct action of the ovary through the folliculin in the blood stream on the cervical mucus.

The few cases presented thus seem to point to a variation in the properties of the cervical mucus dependent apparently on ovarian activity. At the same time proper specimens are hard to obtain, the work requires much time, and there are so many pitfalls that one must hesitate to make more than tentative assumptions. I present these few cases only because they may point the way for further investigations and may prevent too hasty and incautious interpretations of the findings obtained by this method of investigation of sterile couples.

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A CLINICAL STUDY OF BLOOD IRON AND HEMOGLOBIN*

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WE ARE approaching this subject with a healthy skepticism, born of the belief that, in spite of the great progress that has been made in hematology during the past few years, we are still surrounded by a mass of doubtful and uncertain data that leaves us far from a simple and satisfactory working basis. One must respect and accept as authoritative the original work of Haden,¹ Osgood and Haskins² and others for their valuable contributions. We cannot but feel that our immediate clinical need is the adoption of simpler methods to remove us from much of the chaos that for the past few years has served to confuse us. We believe that for many years at least the estimation of the number of red blood cells and the hemoglobin per given quantity of blood, as well as their relationship to each other, will constitute the chief desideratum in hematologic studies. One must also give greater thought to the question of iron in its relationship to hemoglobin.

It seems unnecessary to further stress the great importance of reporting the amount of hemoglobin in grams per 100 c.c. of blood, but a citation of a few of the existing errors will not be amiss. For instance one of the Sahli instruments is so graduated to indicate 100 per cent hemoglobin as containing 17.2 gm., one of the Dare instruments to contain 13.8 gm. of hemoglobin, representing a difference of more than 20 per cent, perhaps better shown by the equation, viz.:

$$17.2 \text{ gm. Sahli} = 100 \text{ per cent}$$

$$17.2 \text{ gm. Dare} = 124 \text{ per cent}$$

It should be known also that there is a wide difference in various Sahli instruments as well as in the Dare instruments. Certainly percentage reports are meaningless and even when accompanied by the name of the method used are not accurate unless such instrument has been standardized with one of the more accurate methods to be mentioned. What should be accepted as a normal average figure for the number of grams of hemoglobin contained in 100 c.c. of blood, and what should be accepted as a normal average figure for the number of red blood cells per cubic millimeter of blood? Personally, we believe that no single figure will serve the purpose with even a fair degree of accuracy. Such figures are ordinarily obtained by taking an average for a large number of so-called normals. In this number we will find a range of from 11.0 to 16.5 (a difference of 34 per cent) gm. of hemoglobin per 100 c.c. of blood, and a range of from 4,000,000 to 6,000,000 (a difference of 34 per cent) for the number of red blood cells per cubic millimeter of blood. Certainly such varia-

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tions are too great to permit of accurate averaging. The question naturally arises as to what is normal blood. An individual may be subjectively healthy and with perfectly normal functional tests of all kinds and still have comparatively low hemoglobin and red cell counts. Such findings are not uncommon among nurses, technicians, secretaries, etc., and such individuals constitute a large percentage of our so-called normal series. Conversely an individual may have advanced heart, kidney, lung, gastrointestinal, or other pathologic conditions with blood examinations much nearer the generally accepted average than the class previously mentioned (Table IV). These individuals are not used in the normal series.

Both the quantity of hemoglobin and the number of red blood cells are variables, depending probably upon age, sex, climate, food, exercise, and barometric pressure. One of us (J. D. McN.) examined the blood of one patient daily for a month and hourly for a day and found the red blood count to vary from 5,128,000 to 6,176,000 and the hemoglobin from 11.3 gm. to 15.3 gm. With such variations why not abolish such terms as "normal averages" and substitute "a normal range" for hemoglobin and red blood cells as we do for the chlorides, sugar, cholesterol, and the nitrogenous products of the blood.

This study includes about one hundred examinations of the blood made upon 81 individuals (Table I). We are unable to explain the difference between the average number of red cells in the normal bloods of our series and the normal bloods reported by others. The fact remains, however, that a count of five million is a rare exception rather than the rule, with a large percentage running from four to four and a half million. Each of these counts, as well as

TABLE I
BLOOD STUDIES IN CASES WITH A VARIETY OF CONDITIONS

CASE	SEX	AGE	DIFFERENT LABORA- TORIES		BLOOD IRON	HEMOGLOBIN DETERMINATIONS			COLOR INDEX HB. R.E.C. $\times 3$	IRON INDEX	DIAGNOSIS
			R.E.C.	R.E.C.		FROM IRON	O. & H.	N.			
1	F	36	4,096	4,340	43.2	12.7	12.0	13.0	0.93	10.5	Normal
2	M	41	4,312	4,070	46.5	13.6	13.6	13.9	0.93	10.3	Normal
3	F	45	4,200		41.5	12.1	12.1		0.87	9.8	Syphilis
4	M	69	4,430	4,310	48.5	14.3	14.3	13.2	0.98	10.9	Cholecystitis
5	F	53	4,232		42.6	12.5	12.5		0.87	10.0	Hypertension
6	F	24	4,240	4,510	39.8	11.6	11.5	11.8	0.82	9.4	Normal
7	M	64	4,536		47.5	13.9	14.0		0.91	10.4	Normal
8	M	54	4,720	4,355	48.4	14.2	14.2	12.9	0.90	10.2	Normal
9	M	45	4,312	4,445	43.0	12.6	12.3	13.1	0.87	10.0	Neurosis
10	M	57	4,304	4,321	43.0	12.6	12.8	13.2	0.87	10.0	Hypertension
11	F	27	4,496	4,300	45.4	13.4	13.3	12.8	0.90	10.1	Hydronephrosis
12	F	34	4,480	4,470	41.6	12.1	12.0	12.3	0.81	9.0	Neurosis
13	F	67	4,680	4,700	47.0	13.8	14.0	13.6	0.88	10.0	Normal
14	F	65	4,408	4,360	42.8	12.5	12.8	13.3	0.84	10.6	Hypertension
15	F	35	4,256	4,320	42.5	12.4	12.4	12.2	0.87	10.0	Surg. menopause
16	M	58	4,208	4,120	43.5	12.8	13.1	12.4	0.90	10.3	Hypertension
17	F	20	4,576	4,520	46.8	13.7	14.0	13.2	0.89	10.4	Normal
18	F	22	4,288	4,350	41.3	12.1	12.3	13.0	0.88	9.8	Normal
19	F	53	4,696	4,363	42.9	12.6	12.8	12.9	0.90	10.2	Normal
20	F	55	4,208	4,376	40.8	11.9	12.1	13.0	0.84	9.5	Arthritis
21	F	47	4,480	4,350	48.0	14.1	14.0	13.3	0.93	10.9	Migraine
22	F	27	4,384	4,350	44.5	13.1	13.4	13.2	0.88	10.3	Normal

hemoglobin determinations, have been made independently by two different workers in different laboratories, using standardized equipment, and discarding results when the two counts did not come within the average limits of error. Furthermore, the work was done by skilled hematologists and not by the usual laboratory technicians. The experience of other reliable laboratories convinces us that in Kentucky at least a normal red cell count of five million is the ex-

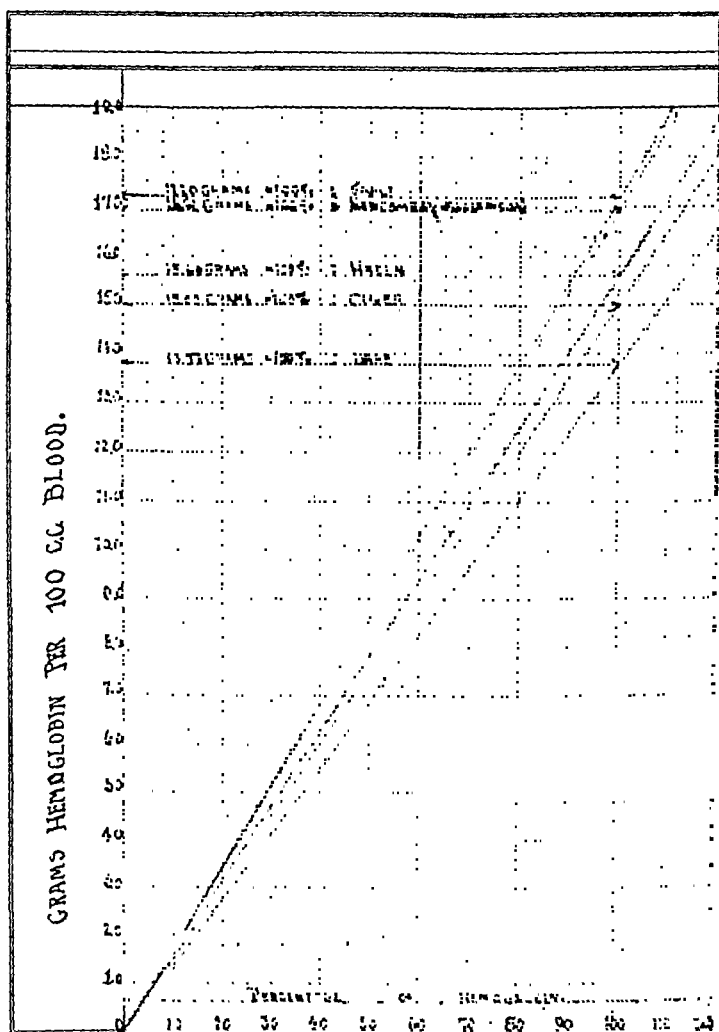


Chart 1.—Comparing hemoglobin from iron (Kennedy) with Osgood and Haskin's method.

ception and the average is from 4.3 to 4.5 million, depending largely upon the source of the normals.

Greater accuracy attends the proper determination of the hemoglobin when the method used has been carefully checked with the accurate oxygen capacity method of Van Slyke,³ or the iron method of Kennedy or Wong. Inaccuracy of various methods has been stressed by Osgood and Haskins,² Karshan and Freeman,⁴ Haden,¹ Lindsay Rice and Sellinger,⁵ Robscheit,⁶ and

others. The chief objection to the acid hematin methods seemed to lie in the time required for development of the color, fading of standards, variation in standard discs, and difficulty in reading small quantities of hemoglobin. The Osgood and Haskins is probably the most accurate of the acid hematin methods provided the standard solution is properly prepared and the proper corrections made for variations in temperature. The Palmer⁷ method is quite accurate if the standard is frequently checked. The photoelectrometer of Sanford and Sheard⁸ and the spectrophotometer of Haden⁹ are apparently accurate methods, checking closely with the oxygen capacity method or the iron method. Although quantitative determinations of iron in the blood were made as early as 1820¹⁰ and at long intervals following that, the most recent by Berman in 1918,¹¹ Brown in 1922,¹² Wong in 1928,¹⁴ and Kennedy in 1927,¹⁵ the method received little attention until its clinical application by Powers and Murphy.¹⁶

Of the methods of Wong¹⁴ and Kennedy¹⁵ the latter points out that Wong, like others, has overlooked the question of dissociation and continued to work with aqueous solutions. The results, however, by the two methods check so very closely that the criticism may well be ignored.

Using Kennedy's¹⁵ method Powers and Murphy¹⁶ found the amount of iron in the blood to be within singularly narrow and standard limits for normal adults. They consider it the most accurate criterion yet devised for evaluating the true status of patients with secondary anemia. In a later article Murphy, Lynch and Howard¹⁷ reported a large number of blood iron determinations in normal individuals, and also in the primary and secondary anemias, as well as a variety of other conditions. For normal young men they found an average of 44.84 and for young women an average of 42.84 mg. per 100 c.c. of blood. Using the same method, we find very close agreement with an average of 44 for all normal cases. As would be expected, the results parallel closely the hemoglobin curve in all cases. They also suggested the term "iron index" (obtained by dividing the mg. of iron in 100 c.c. of blood, by the number of red blood cells in millions per 1 c.mm. of blood) to represent the amount of iron in the individual red cell. They found for this an average of 8.4 (strictly speaking $8.4 \text{ mg.} \times 10^{-11}$) for normals, and following in a general way the color index curve. Since our red blood counts are lower on an average, the average normal index is higher, being as consistently 10 in our series as the 8.4 of their series (Tables I and IV).

Both Kennedy¹⁵ and Wong¹⁴ have used the iron content of the blood for accurately determining the amount of hemoglobin. When it is possible to obtain results for the iron content of tissue or fluids, that are accurate to the third decimal and with the molecular weight of the hemoglobin molecule known (though the structure may be in doubt), it becomes immediately apparent that the quantity of iron expressed in milligrams divided by the iron portion of the hemoglobin molecule 0.334 by Wong and 0.334 by Kennedy) can scarcely be exceeded for accuracy when properly decimated to represent the quantity of hemoglobin in gm. per 100 c.c. of blood. The work of Riecker,¹⁸ however, seems to prove that in normal individuals there is an average of 1.1 mg. (± 0.022) of

iron in the blood serum, and this quantity is increased in pernicious anemia particularly and slightly decreased in certain other conditions. In our study we have assumed (possibly wrongly) that cognizance of this nonhemoglobin iron

TABLE II
PRIMARY DISEASES OF THE BLOOD

CASE	SEX	AGE	DIFFERENT LABORA- TORIES		BLOOD IRON	IRON INDEX	COLOR INDEX HB. R.B.C. × 3	HEMOGLOBIN DETERMINATIONS			DIAGNOSIS
			R.B.C.	R.B.C.				N.*	O. & H.*	FROM IRON	
29	M	57	2,896	2,970	30.0	10.9	0.90	8.3	8.3	8.7	Acute myelogenous leucemia (autopsy)
33	F	61	3,592	3,400	30.8	8.6	0.75	9.2	8.7	8.9	Banti's disease
33	F	61	3,880	4,260	34.5	8.9	0.78	13.0	9.9	10.0	After splenectomy
35	M	8	2,240		22.2	9.9	0.85	13.6	6.1	6.4	Sickle cell anemia
44	F	59	4,328	4,450	45.5	10.6	0.91		14.4	13.4	Pernicious anemia (remission)
45	F	71	4,032	4,100	42.3	10.6	0.91	12.8	12.6	12.4	Pernicious anemia (remission)
67	M	75	992	1,020	14.3	14.4	1.20	5.5	4.0	4.0	Pernicious anemia
70	M	60	1,792	1,760	21.9	12.2	1.05	4.8	6.4	6.3	Pernicious anemia
71	M	65	3,520	3,000	39.0	11.0	0.97	7.07	11.6	11.4	Pernicious anemia
72	M	55	1,994	1,960	29.5	14.3	1.29	5.90	8.8	8.6	Pernicious anemia
76	F		1,104		18.4	16.6	1.41		5.5	5.2	Pernicious anemia
73	F	18	2,688	2,850	23.3	8.7	0.74	5.5	6.9	6.7	Chlorosis
37	F	51	3,280	3,490	32.0	8.3	0.72	9.0	9.0	9.3	Chronic lymphatic leucemia, lobar pneumonia
77	F	56	7,504	7,420	75.5	10.0	0.88	21.0	21.8	22.3	Polycythemia vera Phenylhydrazine gr. IV daily Determinations weekly intervals
			7,840	7,620	72.5	9.3	0.81	21.0	21.0	21.4	
			7,968	7,820	80.6	10.2	0.90	22.0	22.8	23.9	
			7,264		70.0	9.7	0.85		20.2	20.7	
			7,200		65.0	9.0	0.80		19.6	19.2	
			6,984		60.6	8.8	0.78		18.2	17.9	
			3,872	3,872	37.8	9.7	0.87	13.5	11.3	11.0	

*N = Newcomer, O & H. = Osgood & Haskins.

Red blood cells expressed in millions per 1 c.c.: Blood iron in milligrams per 100 c.c.: Hemoglobin in gm. per 100 c.c.: Iron Index = Fe. (gm.) ÷ R.B.C. (millions): Color Index = Hb. (gm.) ÷ R.B.C. (millions) × 3: Hemoglobin from iron calculated viz.: Mg. blood iron per 100 c.c. - mg. serum iron × 3.

TABLE III
DISEASES WITH ABNORMAL BLOOD

CASE	SEX	AGE	DIFFERENT LABORA- TORIES		BLOOD IRON	IRON INDEX	COLOR INDEX HB. R.B.C. × 3	HEMOGLOBIN DETERMINATIONS			DIAGNOSIS
			R.B.C.	R.B.C.				N.*	O. & H.*	FROM IRON	
24	F	43	3,904		35.4	9.0	0.78		10.3	10.3	Nutritional edema
31	F	15	2,728	2,770	27.7	10.0	0.87	7.5	8.0	8.0	Hemorrhagic nephritis (autopsy)
34	F	60	3,784	3,784	37.5	9.9	0.87	11.6	11.1	11.0	Duodenal ulcer; py- loric obstruc.
38	F	2 wk.	2,984		39.5	13.7	1.16		11.0	11.5	Hydrocephalus; sec- ondary anemia
40	M	40	3,520	3,520	35.1	10.0	0.87	12.0	10.0	10.2	Cerebrospinal syphilis —secondary stage
74	M	70	4,640	4,410	46.1	10.0	0.87	14.0	13.9	13.5	Nephritis; pneumonia
50	F	24	3,840	3,810	37.4	9.7	0.93	12.4	10.4	10.9	Colitis; focal infec- tion
58	F	37	3,856	4,300	40.0	10.5	0.90	12.8	11.7	11.7	Colitis; neurosis
68	F	53	3,896	3,910	38.8	10.0	0.87	12.0	11.6	11.3	Secondary anemia
80	M	47	3,520	3,525	37.0	10.5	0.91	11.5	11.0	10.8	Duodenal ulcer; bleeding

*N = Newcomer. O. & H. = Osgood & Haskins.
Footnote same as in Tables II and IV.

TABLE IV
DISEASES WITH NORMAL BLOOD

CASE	SEX	AGE	DIFFERENT LABORA- TORIES		BLOOD IRON	IRON INDEX	COLOR INDEX HB.	HEMOGLOBIN DETERMINATIONS			DIAGNOSIS
			R.B.C.	R.B.C.				N.*	O. & H.*	FROM IRON	
23	M	47	4,800		46.7	9.7	0.85		13.8	13.7	Renal calculus Bronchiectasis
25	F	60		4,470	55.0	12.3	1.11	13.1		16.5	Adeno-carcinoma ovary
32	F	55	4,112	4,270	43.0	10.4	0.91	12.2	12.2	12.6	Arthritis Focal infection
36	M	12	4,048		39.9	9.8	0.86		11.9	11.7	Typhoid fever
39	F	55	4,400		42.8	9.7	0.85		12.0	12.5	Myocarditis, mitral insuff., focal infect.
41	M	52	5,696	4,940	55.5	9.7	0.85	14.9	15.9	16.3	Hypertension Gastroenterostomy
42	F	27	4,128	4,332	41.4	10.0	0.87	12.9	12.0	12.0	Thromboangiitis oblitrans
43	F	28	4,280	4,200	40.0	9.5	0.81	12.7	12.2	11.7	Trichiniasis
46	M	68	4,304	4,480	41.5	9.6	0.84	13.1	11.3	12.1	Cirrhosis of liver Myocarditis
47	F	65	4,800		49.0	10.2	0.90		14.5	14.4	Endarteritis obliter- ans, gangrene
48	F	43	4,160		41.6	10.0	0.87		12.2	12.2	Cholecystitis Mild hypothyroidism
51	F	60	4,224	4,280	42.2	10.0	0.87	12.8	12.0	12.3	Acoustic tumor
52	F	29	4,280	4,320	40.4	9.4	0.82	12.9	12.0	11.8	Pelvic pathology
53	M	48	5,120	5,500	53.0	10.3	0.90	13.5	15.4	15.6	Diverticulitis colitis
54	F	51	4,608	4,330	46.5	10.0	0.87	13.5	13.6	13.6	Recurrent hyperthy- roidism
55	M	50	4,488		45.1	10.5	0.88	14.6	13.2	13.2	Duodenal ulcer
57	M	58	4,728	5,510	48.8	10.4	0.90	15.3	14.4	14.3	Duodenal ulcer
59	M	20	5,056	4,480	51.0	10.2	0.88	14.1	15.3	15.0	Chronic nephritis
60	F	75	4,200		42.8	10.0	0.90		12.8	12.5	Arthritis
62	F	32	4,480	4,340	45.5	10.1	0.90	12.9	13.6	13.4	Arthritis
63	F	58	4,392	4,370	43.0	10.0	0.85	13.0	13.0	12.6	Auricular fibrillation
64	M	77	4,336	4,321	41.0	9.5	0.81	13.0	12.0	12.0	Auricular fibrillation, diabetes,
65	M	34	4,640	4,485	46.6	10.0	0.87	13.3	14.0	13.7	Duodenal stasis neurosis
66	F	61	4,480	4,350	45.4	10.0	0.88	12.8	13.4	13.3	Uterine fibroid Cholecystitis
69	F	53	4,592	4,430	45.0	10.0	0.87	13.2	13.3	13.2	Arterial hypertension
74	M	70	4,640	4,410	46.1	10.0	0.87	14.0	13.9	13.5	Nephritis, pneumonia (death in 36 hr.)
75	F	29	4,384	4,500	43.8	10.0	0.90	13.2	13.2	12.8	Cerebral lesion
Average			4,514	4,515	45.2	10.0	0.877	13.38	13.19	13.28	

*N = Newcomer. O & H. = Osgood & Haskins.

Footnote same as in Tables II and IV.

has been ignored in the methods of Kennedy and Wong, and we have, therefore, subtracted 1 mg. from the total iron content of the blood and then multiplied the result by 0.3 (equal to dividing by $0.333\frac{1}{3}$, instead of the 0.334 and 0.335 used by Kennedy and Wong respectively). While this method makes a difference of less than 2 per cent it has been used with the belief that accuracy was increased to this extent. The oxygen capacity method of Van Slyke² compares so very closely with both the Wong¹⁴ and Kennedy¹⁵ methods that little or no doubt should be entertained as to the accuracy of any of the three methods. Contrary to the general belief we consider both iron methods quite simple and easily completed in about fifteen minutes. We prefer, however, the

Kennedy method¹⁵ because it allows time for other work while the iron mixture is being digested, and, furthermore, the colorimetric readings are definitely sharper. Any of the three methods may be safely used for standardizing hemoglobinometers and checking the accuracy of other methods.

In our experience, as well as in the experience of others, the Osgood and Haskins² acid hematin method gives results closely paralleling the results of

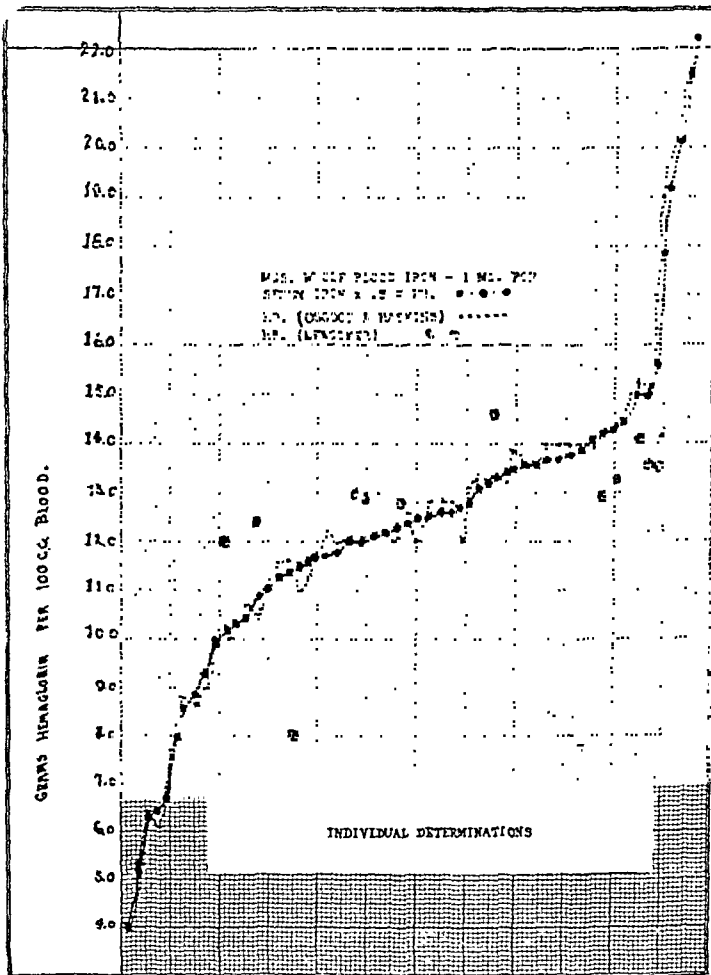


Chart 2.—Showing variation in grams of hemoglobin per 100 c.c. of blood for 100 per cent readings. May also be used for quickly converting percentage into grams.

any of the three standard methods. We believe, therefore, that a determination of the iron content of the blood, the iron content of the individual cell, and the determination of the hemoglobin by the iron method are all most valuable additions to our knowledge of hematology. They assume no averages and deal directly with the number of red blood cells and the hemoglobin found in the individual specimen. Of similar importance is the determination of the amount of hemoglobin in the individual cell. This is done by dividing the hemoglobin in grams per 100 c.c. of blood by the red cells in millions per c.mm.

of blood. The result is expressed in grams times 10^{-12} , or in billionths of a milligram per red cell, according to the following formula:

$$\frac{\text{Hb. in gm.} \times 10}{\text{red cells in millions}}$$

Example: R. B. C. 3,500,000, Hb. 10.5 gm.

$$\frac{10.5 \times 10}{3.5} = \frac{105}{3.5} = 30 \text{ billionths of a mg. per R. B. C. or } 30 \times 10^{-9} \text{ mg.}$$

The color index, determined in our series by the method of Cullen,¹⁹ cannot be quite so accurate, since it assumes five million red cells to be normal,

TABLE V

A ROUTINE REPORT OF A BLOOD EXAMINATION (MARTIN & MCNEILL LABORATORIES)

BLOOD EXAMINATION

R.B.C.	2,896,000-2,970,000 (repeated)
Hemoglobin (Osgood & Haskins)	9.0 gm. per 100 c.c. blood (normal about 15)*
Average amount per red cell	31 billionths of a mg. (normal about 30)*
Blood iron	30 mg. per 100 c.c. blood (normal about 45)
Iron index	10.3
W.B.C.	20,050-21,050 (repeated)

DIFFERENTIAL

	<i>Romanowsky Stain</i>	<i>Supravital Stain</i>	
	<i>Per cent</i>	<i>Per cent</i>	<i>No. per c.mm.</i>
Myeloblasts	45.0	69.5	13,900
Promyelocytes	1.0	2.0	400
Neutrophilic myelocytes	1.0	4.5	900
Eosinophilic myelocytes	0.0	0.0	0
Basophilic myelocytes	0.0	0.5	100
Metamyelocytes	1.0	1.5	300
P. M. neutrophiles	9.5	7.5	1,500
P. M. eosinophiles	0.0	0.0	0
P. M. basophiles	0.0	0.5	100
Monocytes	1.5	6.0	1,200
Lymphocytes	2.0	7.0	1,400
Immature lymphocytes	0.0	1.0	200
Smudges	39.0	0.0	0
	100.0	100.0	20,000

Platelet count	25,000
Arneth's index	89 (normal about 60)
Reticulocytes	10%
Malarial parasites	None

The number of red cells is considerably reduced, but the average amount of hemoglobin per red cell is normal, due to an increase in the average size of the cells. Many of the large cells are polychromatic, and a number of normoblasts and megaloblasts are present. There is very little distortion.

Thirty-nine per cent of the white cells are so fragile as to be destroyed in pulling a smear. These fragile cells are identified in the supravital preparation as myeloblasts. These cells have a large round nucleus with several nucleoli and a basophilic cytoplasm. All gradations between these nongranular cells and the typical adult neutrophile can be found.

Impression: Myeloblastic leucemia.

*The percentage of hemoglobin of this patient varies according to the normal standard of hemoglobin selected—for instance, with the Sahli standard it is 52% (color index 0.9), and with the Dare standard it is 66% (color index 1.2).

and this factor enters into the equation. The results, however, conform closely with our general knowledge of what this should be, namely, high in pernicious anemia and low in secondary anemia.

It is our belief that a properly reported blood examination should contain the following data: (1) sufficient counts of the red blood cells and the white blood cells until they check within the accepted limits of error. (2) Hemoglobin determinations by the iron method of Wong¹¹ or Kennedy,¹² or the acid hematin method of Osgood and Haskins,² (3) the average amount of hemoglobin per red cell, (4) the blood iron in milligrams per 100 c.c. of blood, and (5) the iron index, (6) a supravital stain of the white cells as well as examination of the stained smear (this we believe should never be omitted and furnishes information not obtainable from the stained smear), (7) a platelet count, (8) reticulocyte count, and (9) interpretation of the findings and explanation of various hemoglobin standards (Table V). Thus it will be seen that a complete hematologic study is far from the simple procedure that has been used for years and impossible of accomplishment in the ten minutes that has been allotted and boasted for it. Most technical procedures in the laboratory have been greatly simplified without affecting their accuracy, but with our increasing knowledge of the blood, the procedure, if accurate, is becoming more difficult and more time-consuming, which is regrettable from many standpoints, the least of which is by no means financial. Because of the time consumed and the skill required it should not be included along with a urinalysis in a so-called routine examination, but accredited the same dignity that is accorded other special procedures.

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BLOOD STUDIES IN HYPERTHYROIDISM*

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GENERAL attention was first drawn to the importance of the blood picture in hyperthyroidism by the exhaustive studies of T. Kocher¹ and his co-workers. References to the blood changes occurring in hyperthyroidism were, however, made before Kocher's studies by Ciuffini² and particularly by L. Caro.³ Kocher considered the blood picture as quite typical, namely a leucopenia, neutropenia, and a lymphocytosis. These observations were confirmed by Crotti,⁴ Müller,⁵ Lampe,⁶ Hertz and Lerman,⁷ and others. Some observers, however, could not quite confirm the so-called "Kocher blood picture," e.g., Plumer in his extensive study of 578 cases. The leucopenia particularly has not been found regularly. If one considers, however, the fact that patients suffering from hyperthyroidism are frequently subject to profuse perspiration and diarrhea, which consequently leads to a certain degree of dehydration and blood concentration, one can easily understand the occasional absence of the leucopenia; in other words the blood concentration may mask the presence of the leucopenia.

The following morphologic blood studies were undertaken originally upon the request of Professor E. W. Archibald, who had made, about ten years ago, the observation, in several cases, that the blood from the thyroid vein contained a much higher percentage of lymphocytes than the blood from the thyroid artery, or the peripheral capillary blood. The difference at that time was calculated in percentages, and it was found in two cases that a percentage of between 30 and 40 in the thyroid artery, and in the peripheral capillary blood was in-

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creased to 75 and even 80 in the blood of the thyroid vein. Samples of blood were taken at the beginning of the operation, immediately upon exposure of the thyroid gland. Professor Archibald desired confirmation of the findings in these few cases. The observation seemed to indicate that a large number of lymphocytes was added to the circulating blood by the fact of its passage through the thyroid gland. With regard to this point the blood of the thyroid artery was compared morphologically with the blood of the thyroid vein in 7 cases; the blood samples were obtained during the operation.

The scope of the investigation was, however, enlarged by me to include an examination of the phagocytic power of the leucocytes, a detailed study of morphologic elements, and also blood examinations upon experimental animals. The phagocytic power of the leucocytes was studied in 6 cases of hyperthyroidism and compared with the normal.

Signs and symptoms of hyperthyroidism were produced experimentally in 3 cats by the injection of thyroxin, and morphologic blood studies made for a period of thirty days and compared with the blood of 3 normal cats.

RESULTS

Erythrocytes and Hemoglobin.—Anemia was not found to be a striking point of the picture, although low normal and slightly subnormal figures are the usual findings. A slight tendency to macrocytosis was usually observed, leading to a color index of 1 or occasionally slightly above (see Table I).

TABLE I
AVERAGE FINDINGS IN 14 CASES OF HYPERTHYROIDISM BEFORE AND AFTER THERAPY

Hb.	82% 11.48 gm.	80% 11.20 gm.
R.B.C.	4,070,000	4,030,000
C.I.	1.02	1.00
W.B.C.	4,660	5,738
Basophiles	13.8 0.3%	5.7 0.1%
Eosinophiles	105.8 2.3%	142.5 2.5%
Myelocytes	0	17.1
Metamyelocytes	0	0.3%
	0	14.25
	0	0.25%
Stab. forms	105.8 2.3%	228 4%
Normal segmented	1,012 22%	1,806.9 31.7%
Hypersegmented	749.8 16.3%	632.7 11.1%
Lymphocytes	2,042.4 44.4%	2,194.5 38.5%
Monocytes	455.4 9.9%	541.5 9.5%
Thromboocytes	213,291	241,333
Remarks	Preoperative	2 weeks postoperative

Total Number of Leucocytes.—The total number of leucocytes was mostly found to be low. Subtotal thyroidectomy usually led to an immediate moderate increase in the leucocyte count, which however returned to the original low level or a slightly higher one within about 2 weeks. The number of leucocytes was found in the differential count to be due to a reduction in granulocytes, especially the neutrophiles.

Basophiles and Eosinophiles.—Although normal in their percentage figures, they show low absolute numbers.

Neutrophiles.—The neutrophilic granulocytes were always found to be diminished, in percentage as well as in their absolute figures. An increased segmentation of the nuclei was a striking feature, leading to a shift to the right (Arneth-Shilling) with slight evidences of degeneration. Immediately after operation and coinciding with the temporary increase in the total number of leucocytes a considerable number of young forms appeared, returning, however, within about two weeks to almost the original formula, irrespective of the clinical condition or basal metabolism rate of the patient.

Lymphocytes.—Lymphocytes always showed a marked, but mostly relative lymphocytosis. There is a very slight relative reduction of the lymphocytosis after operation.

Monocytes.—A high percentage of monocytes was usually present before and after the operation, the presence of a monocytosis was recently pointed out by Hertz and Lerman⁷ (see Table I).

Thrombocytes.—The thrombocytes usually showed low normal or slightly subnormal numbers. After operation there is a temporary increase in the number of thrombocytes, returning, however to the original level in about two weeks.

COMPARISON BETWEEN THYROID ARTERY AND THYROID VEIN

Six of the seven cases in which the blood of the thyroid artery and thyroid vein was compared were cases of hyperthyroidism with definite toxic evidences, while one of the seven cases was a case of nontoxic goiter (Tables II and III show findings in these cases).

There is a definite increase in the total number of leucocytes in the thyroid vein; this increase is due almost entirely to an increased number of lymphocytes, on the average an increase of $33\frac{1}{3}$ per cent in the blood of the thyroid vein. Only the case with the nontoxic goiter shows an increase in the leucocyte count of the thyroid vein, which is so small, that one may have to consider it within the limit of error.

The morphology of the blood of the thyroid artery compared with that of the peripheral blood shows practically no difference. Considering the findings of pathologists, of a general lymphatic hyperplasia in hyperthyroidism with lymphatic foci in the thyroid gland itself, we can assume that this increase of lymphocytes in the thyroid vein must be due to an actual formation of lymphocytes in the thyroid gland.

Phagocytic Ability of the Leucocytes.—The phagocytic power of the leucocytes was studied by an adoption of Hamburger's method.⁸ About 20 c.c. of oxalated blood was allowed to stand in test tubes, kept in an incubator at 37° ,

TABLE II
BLOOD FROM THYROID ARTERY

NO. OF CASE	3	4	5	6	8	9	10	AVERAGE
Hb.	86% 12.04	80% 11.20	83% 11.62	83% 11.62	78% 10.92	79% 11.06	92% 12.88	83% 11.62
R.B.C.	4,310,000	3,000,000	4,165,000	4,100,000	3,880,000	3,920,000	4,710,000	4,140,000
C.I.	1	1.02	1.01	1.01	1.02	1.01	0.97	1.01
W.B.C.	5,600	4,300	5,300	6,100	5,100	4,720	6,120	5,320
Basophiles	0	0	0	61	0	0	0	5
Eosinophiles	0	0	0	1%	0	0	0	0.1%
Myelocytes	168	86	106	122	153	94	129	116.6
	3%	2%	2%	2%	3%	2%	2%	2.2%
Metamyelocytes	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0
Stab. forms	0	0	0	0	0	0	0	0
	168	86	159	122	102	94	183	127
Normal segmented	3%	2%	3%	2%	2%	2%	3%	2.4%
	1,400	1,032	1,378	1,403	1,071	987	2,196	1,330.3
Hypersegmented	25%	24%	26%	23%	21%	21%	36%	25.1%
	1,008	774	954	1,159	1,020	846	549	906.3
Lymphocytes	18%	18%	18%	19%	20%	18%	9%	17.1%
	2,408	1,935	2,173	2,623	2,301	2,209	2,684	2,332
Monocytes	43%	45%	41%	43%	45%	47%	44%	44%
	448	387	530	610	459	470	366	466.4
Thrombocytes	8%	9%	10%	10%	9%	10%	6%	8.8%
	235,000	165,000	241,000	192,000	210,000	212,000	291,000	220,857
Remarks							Nontoxic goiter	

TABLE III
BLOOD FROM THYROID VEIN

NO. OF CASE		3	4	5	6	8	9	10	AVERAGE
Hb.	% Gm.								
R.B.C.		4,210,000	4,030,000	1,015,000	1,130,000	3,850,000	3,790,000	4,630,000	4,097,000
C.L.		1.01		1.01	1	1.01		0.97	1.02
W.B.C.		7,000	6,000	6,900	7,800	6,200	5,800	6,670	6,632
Basophiles		0	0	69	78	62	0	0	26
		0	0	1%	1%	1%	0	0	0.4%
Eosinophiles		140	120	207	136	124	116	198	145.2
		2%	2%	3%	2%	2%	2%	3%	2.2%
Myelocytes		0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0
Metamyelocytes		0	0	0	0	0	0	0	0
Stab. forms		140	60	138	78	62	53	264	112.2
		2%	1%	2%	1%	1%	1%	4%	1.7%
Normal segmented		1,470	1,200	1,380	1,482	1,178	1,160	2,112	1,419
		21%	20%	20%	19%	19%	20%	32%	21.5%
Hypersgmented		1,190	900	1,104	1,326	1,054	812	528	976.8
		17%	15%	16%	17%	17%	14%	8%	14.8%
Lymphocytes		3,430	3,120	3,381	3,900	3,162	3,132	3,168	3,333
		49%	52%	49%	50%	51%	54%	48%	50.5%
Monocytes		560	600	621	750	620	522	330	574.2
		8%	10%	9%	10%	10%	9%	5%	8.7%
Thrombocytes		217,000	190,000	218,000	185,000	236,000	224,000	278,000	222,000
Remarks								Nontoxic goiter	

for four hours. The supernatant serum was first carefully removed, and then the leucocytes which accumulate on top of the erythrocytes. The leucocytes were then placed in normal saline, washed repeatedly with normal saline changes, and finally a suspension in normal saline was made. Five-tenths cubic centimeters of that suspension was brought into contact with 5 c.c. of the serum from the same individual and incubated for one hour at a temperature of 37° C. After one hour, 0.5 c.c. of india ink was added to the suspension and incubated again for one hour. The tubes were carefully shaken every ten minutes to maintain an equal suspension. From the incubator the tubes were then transferred to an ice box for two hours, and then smears made and stained by Pappenheim's method (Jenner and Giemsa combination). In the smears 500 neutrophilic cells were counted and the number of cells containing carbon particles were marked.

TABLE IV

CASE	PHAGOCYTOSIS OF NEUTROPHILES INCUBATED IN THEIR OWN SERUM	NO. OF CASE	PHAGOCYTOSIS OF NEUTROPHILES INCUBATED IN THEIR OWN SERUM
Normal	35 per cent	2	10 per cent
Normal	38 per cent	3	12 per cent
Normal	34 per cent	5	14 per cent
Normal	32 per cent	8	12 per cent
Normal	39 per cent	13	12 per cent
Normal	37 per cent	15	14 per cent
Average	35.8 per cent	Average	12.3 per cent

The neutrophiles of the normal individuals showed an average of 35.8 per cent of the cells having stored carbon particles, while only 12.3 per cent of the neutrophiles of the patients with hyperthyroidism showed storage of carbon particles (see Table IV). The question arose now whether we are dealing with a faulty type of leucocytes in hyperthyroidism, or whether the reduction in phagocytosis was due to some factor in the serum of the patient. For that pur-

TABLE V

NO. OF CASE	PHAGOCYTOSIS OF NEUTROPHILES INCUBATED IN NORMAL SERUM (GROUP IV) %
2	28 per cent
3	30 per cent
5	32 per cent
8	27 per cent
13	28 per cent
15	29 per cent
Average	29 per cent

pose the corpuscles of the patients were incubated in the serum of a normal individual (Group IV, to exclude possible precipitations) and the corpuscles of the normal individual (Group IV) were incubated in the sera of the patients. The neutrophilic cells of the patients, previously showing an average of 12.3 per cent phagocytosis, after incubation with the normal serum showed an average phagocytosis of 29 per cent. The normal corpuscles, originally having a phago-

cytic ability of 35 per cent, after incubation with the sera of the patients showed only 13.5 per cent phagocytosis (see Tables V and VI).

From these figures it can be seen that the phagocytic ability of the neutrophils in hyperthyroidism is definitely diminished, but this reduction of phagocytosis is not due to a faulty cell, but to the influence of the serum of the patient with hyperthyroidism, whether we are dealing with a deficient serum or whether there is a toxic inhibitory substance in the serum cannot be explained by the above data.

Experimental Hyperthyroidism.—Thyroxin was found to produce after 8 to 10 daily intramuscular injections of 1 mg. per kilo of body weight, definite

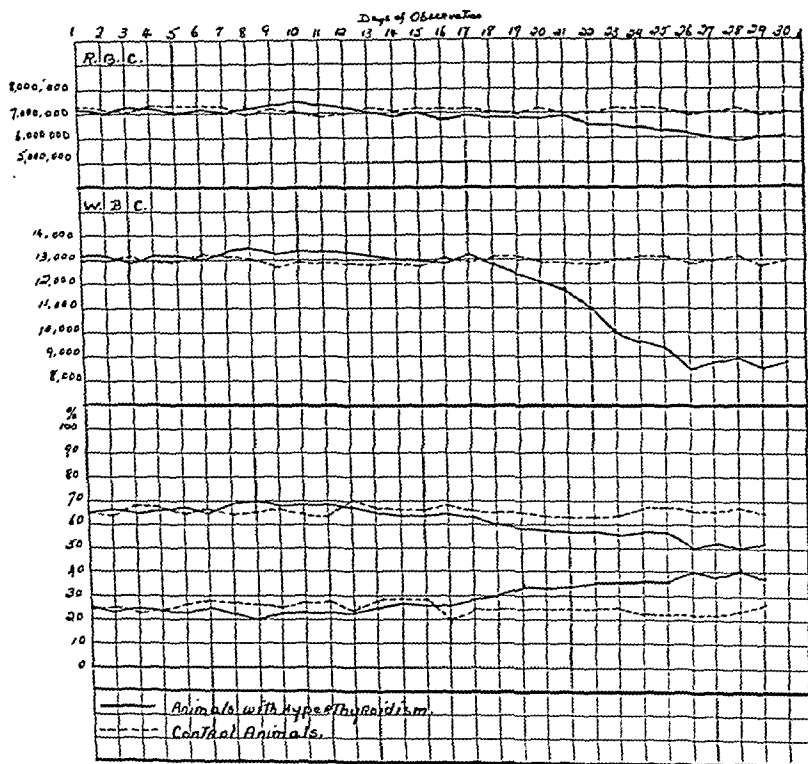


Chart I

signs of hyperthyroidism. Six cats were used for these experiments, 3 of them were given thyroxin, and 3 were used as controls. Daily blood studies were made on all animals for thirty days. The thyroxin injections were started on the seventh day and given daily until definite toxic signs developed (loss of weight, restlessness, diarrhea, etc.), then one or two injections were given weekly.

Erythrocytes.—Erythrocytes showed only slight changes. After a very slight initial rise during the third and fourth day after the injection, the erythrocytes came gradually down to a somewhat lower level during the third week after the injections had been started, leading to a slight degree of anemia. The control animals showed no changes.

TABLE VI

SERUM NO.	PHAGOCYTOSIS OF NORMAL NEUTROPHILES (GROUP IV) INCUBATED IN PATIENTS' SERUM
2	12 per cent
3	15 per cent
5	11 per cent
8	12 per cent
13	16 per cent
15	15 per cent
Average	13.5 per cent

Leucocytes.—The total number of leucocytes remained unchanged for about ten days after the injections were started, then they came gradually down to a definitely lower level. The differential count showed this reduction of leucocytes to be due to a reduction of the granulocytes. The lymphocytes showed coinciding with the reduction of the granulocytes, a mostly relative increase. The same is true of the monocytes. (Chart I shows the average percentage figures during these observations.)

RÉSUMÉ AND CONCLUSIONS

Kocher's blood picture was found to be present in practically all cases of hyperthyroidism. In addition as a striking observation an increase in the segmentation of the neutrophiles was noted, a shift to the right. Although the shift to the right may not be specific for a thyroid disturbance, just as Kocher's blood picture is not specific, it must still be considered as a considerable aid in the diagnosis, since it is not frequently observed in other pathologic conditions. The erythrocytes and thrombocytes are not strikingly diminished, but low normal or slightly subnormal figures are usually present. In other words the whole blood picture is that of a definitely depressed myeloid system. The same is apparently true in experimental hyperthyroidism. The diminished phagocytosis of the neutrophiles, being apparently due to an abnormal serum, together with the whole picture of myeloid depression all speak rather for a toxic influence of the thyroid gland. The lymphocytosis although mostly relative, is also absolute. Whether we are dealing with a general compensatory proliferation of the lymphatic tissue, or whether the proliferation is specific in the pathogenesis of the condition, cannot be decided so far. The presence of the proliferation, however, is definite and quite apparent in the comparison of the thyroid artery and the thyroid vein. I would like here to refer to the work of Hellmann, Heiber, Schlemmer, and others who believe the germinal centers of the lymphatic tissue to be a protective mechanism in the distribution of poisons and toxins. Aschoff in his excellent dissertation on the lymphatic organs⁹ does not quite accept this view, since there is not enough proof for the statements; he accepts, however, the possibility of such a protective mechanism.

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MONILIASIS OF THE BILIARY TRACT*

REPORT OF CASE

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MONILIASIS is a disease which is caused by the *Monilia* fungi. There are over thirty species composing the *Monilia* genus.

Most of the organisms of the *Monilia* genus are widely distributed in nature and can be found on vegetables, fruit, wood, dead leaves, etc.

The name *Monilia* was first given by Hill in 1751 when he described a genus of fungi consisting of a pedicle supporting a number of naked seeds arranged together in a series like the beads of a necklace. Langenbeck in 1839 described the organism of a fungus found in the patches of Thrush in a patient who died from typhus and came to autopsy. Hansen in 1851 studied the fungi from rotting wood and found that in beer wort it produced abundant yeast cells which could produce a mycoderma skin-like infection in which the individual cells again elongated into a mycelium. But to Castellani we are indebted for his tireless investigation and classification of the members of the *Monilia* genus. He defines and describes this genus as follows:

"Oosporaceae, possessing in situ budding forms and mycelial threads which latter are often long and branched; in culture mostly budding forms, but sometimes filaments, in which thallospores of the blastospore type are formed. It ferments dextrose and often other carbohydrate media producing gas."

Castellani classifies the species according to color and fermentation as follows:

White, yellow, pink or red, and black.

Biochemically there are twelve groups:

1. Balcanica Group: gas produced in dextrose only.
2. Krusei Group: gas produced in dextrose and levulose.
3. Pinoyi Group: gas produced in dextrose, levulose, and maltose.
4. Metalondinensis Group: gas produced in dextrose, levulose, maltose, and galactose.
5. Tropicis Group: gas produced in dextrose, levulose, maltose, galactose, and saccharose.

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6. Rhoi Group: gas produced in dextrose, levulose, galactose, and saccharose.
7. Bronchialis Group: gas produced in dextrose, levulose, maltose, and saccharose.
8. Guillermondi Group: gas produced in dextrose, levulose, saccharose, and raffinose.
9. Macedoniosis Group: gas produced in dextrose, levulose, galactose, saccharose, and inulin.
10. Pseudotropicalis Castellani Group: gas produced in lactose in addition to other carbohydrates.
11. Pseudolondiniosis Group: gas produced in lactose in addition to other carbohydrates, but not in lactose.
12. Zeylanica Group: no gas produced in any sugar.

The organisms vary in virulence and pathogenicity depending upon the species of the genus. In the human body they may be found on the skin surface, on the normal mucosa of the mouth and of the upper respiratory and the gastrointestinal tracts.

The *Monilia* genus has been shown to be the offensive organism of a number of *clinicopathologic conditions of the skin, mucous membranes of the mouth, vagina, lung, and intestine.*

Shelmire in 1925 summarized comprehensively cutaneous moniliasis under the heading "Thrush Infection of the Skin." Castellani gives a detail description of moniliasis involving the gastrointestinal tract, claiming that sprue is caused by *Monilia*. He also describes a case of purulent vaginitis caused by a *Monilia* genus.

It will be interesting to note that the etiology of sprue is still in a stage of medical controversy, though investigators like Ashford, Castellani, and Anderson have attempted to prove that *Monilia psilosis* is the offensive organism.

Moniliasis of the biliary tract has not been mentioned by the above investigators, neither has there been any article written on this subject.

However, in the course of my private practice I came across a case which I diagnosed clinically as "moniliasis of the biliary tract." The patient, female, aged forty-four years, resided in Connecticut for the past twenty-one years. While she appeared quite robust and well nourished, yet she complained of severe pain across her back, in her right shoulder and hip. She suffered with these pains for the past four years. Physical examination showed negative findings except for a slightly enlarged spleen and a tender mass in the right upper quadrant which was thought to be the gallbladder. The liver and the kidneys were not palpable. There was dryness in the mouth, but the tongue was clear. In intestinal moniliasis the tongue is coated, sore, and beefy. In this patient the bowels were constipated, and the stools were small in volume, hard and dark in color, while in intestinal moniliasis there is a pale, voluminous, foamy diarrhea. Blood Wassermann, urine, and routine blood work were negative. This patient had chills, fever, sweat on several occasions although smears for malaria, and blood cultures were negative.

The gallbladder was drained for diagnostic purposes. Before the sterile Rehfuess tube was inserted cultures were taken from the mouth, pharynx, and teeth. The result of these cultures was negative for fungi.

A sample of "B" bile was sent to the Connecticut State Laboratory for bacteriologic examination. The report showed that the bile contained *B. coli*, nonhemolytic streptococci, and yeast. On the culture the yeastlike form appeared in large amounts and was similar to *Monilia* morphologically. A portion of this culture was sent to the State Laboratory of Hygiene, Madison, Wisconsin. Doctor W. D. Stovall was kind enough to send a résumé of his report:

"Acid and gas in glucose, levulose, mannose. No fermentation on maltose, galactose, or saccharose. There is a distinct pellicle on the sugars which are fermented by this organism. Colony formation on malt agar was dull, dry and flat with mycelium production in forty-eight hours. This organism corresponds to the cultural relations of several strains of *mycoderma* which we have studied. It also corresponds to the cultural characteristics which we obtained when we studied *M.° Krusei* type."

The bile microscopically showed motile bacteria, bile crystals and pigments, fatty débris, epithelial cells and shreds.

The patient's gallbladder was drained for six consecutive weeks. Internally she was given heavy doses of potassium iodide and hexamethylamine. She was on a cholesterin poor diet.

It was interesting to note that the bile became free from fungi and bacteria, though it showed a few crystals and bile pigments. The patient has been relieved of backache and pain in the joints for over a year.

CONCLUSIONS

A case of moniliasis of the biliary tract is presented. The diagnosis was made by finding the fungus in the bile and on culture.

The treatment consisted of biliary drainage, potassium iodide, and hexamethylamine internally, and a cholesterin poor diet.

Patient has been symptom-free for over a year.

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STUDIES ON RUBBER GLOVE STERILIZATION AND THE USE OF STERILITY INDICATORS*

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FROM the beginnings of aseptic surgery the problem of adequate sterilization of the surgeon's hands has been of major importance. It soon became apparent that mechanical cleansing and chemical disinfection did not suffice, and trials were made of covering the hands with various substances. Methods,¹ such as the use of waxy materials or of cloth gloves impregnated with collodion or liquid paraffin, were devised. Rubber gloves were introduced into surgery by Halstead² in 1889. Their use was recommended by Robb in a textbook published in 1894, and they have been almost universally adopted since that time.

Rubber gloves are commonly sterilized by steam under pressure. This method has the advantage of being simple and efficacious, although it has not been standardized. Different practices are employed in wrapping the gloves, in stacking them in the autoclave, and in the times and pressures utilized for sterilization. The life of the gloves is relatively short, even with the best of care, as exposure to steam under pressure quickly reduces their strength and lessens their elasticity. It may easily be seen that any undue excess of steam pressure or time of sterilization will shorten the useful life of the gloves even more than is necessary.

The experiments to be reported, were planned in order to determine the minimum lengths of time that would be required, at various pressures, to completely sterilize heavily contaminated rubber gloves which had been wrapped in a uniform manner. At the same time an assay was made of the practical value of two types of sterilization indicators which are sold commercially. The indicators used were the Diack† and the Sterilometer.‡

The Diack is a small sealed glass tube containing a pellet of reddish wax-like material. When a certain temperature is reached, the enclosed pellet melts and changes color almost immediately. The temperature required for this change was found to be approximately 121° C., which corresponds to a little over 15 pounds of steam pressure. The complete melting of a Diack which has been placed near the center of a package of material to be autoclaved, is assumed to be indicative of the sterility of the material in question.

The Sterilometer is a strip of heavy paper on which has been printed a small figure representing a thermometer, the bulb and stem consisting of two sections, each of which is composed of a special cream-colored ink. On exposure to heat, these inks darken gradually. Complete blackening of the left-hand section, which is represented by the bulb and part of the stem, is said to

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†From A. W. Diack, 5533 Woodward Avenue, Detroit, Michigan.

‡From the Sterilometer Laboratories, 2308 Victoria Avenue, Los Angeles, Calif.

indicate the maintenance of a temperature of 230° F. for a minimum of approximately fifteen minutes, while the development of a corresponding color in the right-hand section means that 250° F. has been maintained for the same period of time. A Sterilometer, which has had both sections completely blackened by being near the center of a package of material that has been autoclaved, is assumed to indicate the complete sterility of the contents of the package in question. The blackening of the left-hand section alone does not necessarily indicate sterility. This section is merely supposed to act as a color standard and to show that the material in question has actually been autoclaved.*

The following organisms were used in the experiments: *Cl. oedematiens*, *Cl. welchii*, *Cl. septicum* (*Vibrio septique*), *Cl. sordellii*, *Cl. sporogenes*, *Cl. histolyticum*, *Cl. tetani*, *Cl. paratubulinum* A, *B. anthracis* and *B. subtilis*. The anaerobes were grown in chopped veal infusion broth for a period of at least two days before use. Microscopically all, save *Cl. welchii* and *Cl. tetani*, showed a prolific formation of spores. Growth was usually apparent in these latter cultures, however, even after they had been heated to 85° C. for fifteen minutes. *B. anthracis* and *B. subtilis* were grown on agar slants, and formed innumerable spores.

The gloves used were the gloves regularly employed in surgery at the Los Angeles County General Hospital. After contamination they were placed one in each side of a regulation glove book, and this was then wrapped in a double thickness muslin glove wrapper about 26 inches square. In most of the experiments four such packages were tied together before autoclaving. This made up a rather bulky bundle containing eight gloves, and sometimes a ninth glove was inserted in one of the glove books. The use of such bundles was an added factor of safety, as the penetration of steam was definitely slower than is the case in general practice, where gloves are wrapped in small packages and carefully stacked so as to facilitate the rapid penetration of steam.

The autoclave used was the instrument employed for the sterilization of bacteriologic supplies at the Los Angeles County General Hospital. This machine was carefully calibrated in respect to pressure and temperature. It was operated throughout, after a few preliminary experiments by one individual. In each experiment the glove bundles were placed as nearly as possible in the same portion of the autoclave. No preliminary vacuum was produced before the beginning of sterilization, but a special method, for quick elimination of the air, was employed in the later experiments. The door of the autoclave was left slightly open, and steam was allowed to escape at full pressure for a period of from one to two minutes. The door was then closed, and the steam pressure, used for sterilization, was attained very rapidly. This introduced an added factor of safety in that the time taken to attain a certain pressure was cut to the minimum, whereas, in general practice, this time may actually be a considerable factor in the process of sterilization.

Most of the experiments were conducted as follows: Two sterile swabs were

*The Sterilometer has recently been manufactured in a new and slightly different form. The ink in the bulb of the thermometer corresponds to that which made up the former left-hand section, while the stem consists entirely of the ink which composed the former right-hand portion. "The stem must turn black until it matches the color of the bulb," is printed in each indicator.

dipped into one of the spore-filled cultures and were then inserted into different fingers of a glove. A Sterilometer and, under certain circumstances, also a Diack were placed in the cuff of the glove. Eight, and sometimes nine, gloves, prepared in this manner, were wrapped as previously indicated and tied into one bundle. They were then autoclaved for a given time and pressure. The packages in the bundle were then opened, and the swabs were removed and cultured. The anaerobes were cultured in chopped veal infusion broth which had recently been boiled for a few minutes and then cooled. The swabs were inserted, rotated, and withdrawn, and sterile vaseline was poured over the surface of the medium. The aerobes were cultured in deep tubes of extract agar which had been melted and cooled to 40° C. Each culture was labeled with the name of the organism, the date, and the position which its swab had occupied in the bundle of gloves, i.e., whether in the outside or inside glove in an outside or inside package. One control was made directly from the original culture of each organism used. The actual Sterilometer was pasted opposite the record of its corresponding pair of cultures, and the condition of the Diack was recorded.

The cultures were incubated for at least four weeks before being discarded. Such a time is open to criticism as we realize that an occasional spore may lie dormant for an almost indefinite period before germinating. Meyer³ recommends incubating for a three months' period when conducting spore resistance tests on *Cl. paratubulinum* A. Meleney and Chatfield,⁴ however, regard fifteen days as an adequate incubation period in tests to check the sterility of surgical catgut, and we have chosen a time lying between the limits recommended above.

Growth in the anaerobic cultures was clearly evidenced by the production of gas, which forced the vaseline away from the surface of the medium. Growth in the aerobic tubes could be seen on the surface of the agar, and also, to a certain extent, in its depth.

A total of 73 experiments involving over 1,200 cultures, exclusive of controls was performed, all of the cultural work being done by the author. In the early experiments a different organism was used to contaminate each glove in the bundle. An effort was made to distribute the different organisms so that, in a series of experiments, each one would be placed near the inside and near the outside of the bundles an approximately equal number of times. It subsequently became evident, however, that *Cl. oedematiens* was by far the most heat-resistant of the organisms being used. In the experiments in which comparable numbers of the different cultures were employed, 14 out of 76 or 18 per cent of the cultures of *Cl. oedematiens* showed growth, whereas growth occurred in only 5 out of 76 or 7 per cent of the cultures of *Cl. sporogenes*, and the percentage of positive cultures of the other organisms was even less. For this reason all the gloves were contaminated with *Cl. oedematiens* in a number of tests which were performed at a later date.

EXPERIMENTAL RESULTS

In performing the experiments a given pressure was selected and a time which was thought to be inadequate was employed at first. This period was

then lengthened progressively, as growth in the cultures developed, until a time was found for each pressure at which all cultures remained sterile. These minimum sterilizing times were checked, with one exception, by at least three experiments, each involving eight or nine different cultures, and, in addition, three experiments in which *Cl. oedematiens* alone was used. This latter test was considered to be the most crucial of all. In two instances *Cl. paratubulinum* A was substituted for *Cl. oedematiens* in these latter experiments. The question arose as to whether dried spores might be more resistant to heat than are spores in the moist state. In answer to this, in at least one of each series of experiments, designed to check the minimum sterilization times at different pressures, dry gloves were contaminated with swabs on which the spores had been dried. These dried spores showed no more resistance to heat than did the corresponding moist spores under like conditions. Table I gives the results of this series of tests with respect to completeness of sterility attained at different times and pressures.

TABLE I
COMPLETENESS OF STERILIZATION ATTAINED AT DIFFERENT TIMES AND PRESSURES
(CONTROL CULTURES OMITTED)

STEAM PRESSURE IN POUNDS	TIME IN MINUTES	NUMBER OF EXPERIMENTS	STERILE CULTURES	CULTURES SHOWING GROWTH
5	20	2	32	2
5	30	9	149	3
5	40	6	96	0
8	30	6	96	0
10	10	1	11	7
10	15	1	16	2
10	20	7	120	0
10	25	7	118	0
15	10	1	15	3
15	15	3	49	5
15	20	3	48	6
15	25	4	66	0
20	10	4	63	3
20	15	7	118	0

An examination of the above table will show that the minimum sterilizing times attained for different pressures, under the experimental conditions employed, were as follows:

5 pounds	40 minutes
8 pounds	30 minutes
10 pounds	20 minutes
15 pounds	25 minutes
20 pounds	15 minutes

It is perfectly evident that a discrepancy appears between the times which were found to be adequate at ten and at fifteen pounds pressure. This discrepancy illustrated the important fact that the pressure recorded in an autoclave is not always a reliable index of sterilization. In order to insure adequate steam penetration the complete removal of air from the chamber is essential. For this purpose most autoclaves are equipped with an automatic chamber-drain valve and steam trap which allow the escape of air and water of condensation, but hold back most of the steam. It is entirely possible for such an

apparatus to get out of adjustment, in which case air may be retained and the temperature in the center of the packages being autoclaved may be raised relatively little. In such an event, when surgical materials are being sterilized, disastrous accidents may occur. As a check to offset this possibility the use of dependable sterilization indicators would seem advisable.

In two experiments, in which gloves were autoclaved at 15 pounds for twenty minutes, growth occurred in several cultures. The Sterilometers, which had been placed in the cuffs of the gloves that were incompletely sterilized, showed only a partial blackening of their left-hand portion and almost no change at all in their right-hand section. This condition of the Sterilometers was evident immediately upon opening the packages, and it gave presumptive evidence that the gloves in question might not have been completely sterilized, which, indeed, turned out to be the case. Furthermore a check of all the tests shows that in no instance did a completely blackened Sterilometer correspond with a glove that proved to have been incompletely sterilized.

The value of the Sterilometer is further illustrated by the following experience, which recently occurred in our laboratory. Several batches of culture media were found to be contaminated after having been autoclaved in the usual routine manner at 15 pounds for twenty minutes. For this reason it seemed advisable to check the functioning of the sterilizer. Two bundles, each containing four glove packages, were made up in the regular manner. A Sterilometer was placed in each glove bundle, and one was also tied on the outside of each bundle. One bundle was autoclaved at 15 pounds for twenty minutes in the exact manner that had been employed for the above sterilization of media. The second bundle was then sterilized in the same manner, with the exception that special care was taken, in this instance, to completely exhaust all air from the chamber of the autoclave.

Fig. 1 is a photograph of the actual Sterilometers used in the above two experiments. On the left are shown the indicators taken from the bundle which had been autoclaved with special care regarding the complete exhaustion of air. These Sterilometers are all either completely or very nearly completely blackened. They are all very much darker than the darkest Sterilometer that was associated with a contaminated glove in any of our experiments, which goes to show that the penetration of steam was sufficient, in this case, to insure complete sterilization of this bundle of gloves. The Sterilometers in the right-hand column were taken from the bundle which had been autoclaved in the manner routinely employed for culture media. The Sterilometer from the outside of the bundle shows an almost complete degree of blackening. The indicators from within the packages, however, are variable and incompletely darkened, demonstrating that the penetration of steam had been insufficient to insure adequate sterilization in this instance. Such a record should serve as a warning that an autoclave is not functioning properly, a state of affairs which might otherwise be entirely overlooked for a considerable period of time.*

*These experiments were subsequently repeated using gloves contaminated with *Cl. oedematis*. Several of the gloves, which were run in the improperly functioning autoclave, and which were associated with incompletely blackened Sterilometers were found to have been incompletely sterilized. Complete sterility was obtained in the case of those gloves which were associated with thoroughly blackened Sterilometers, a state of affairs which occurred only when all the air was exhausted from the autoclave.

The value of an indicator, such as the Diack, appears to be more problematical. In the experiments summarized in Table I, a melted Diack was always associated with complete sterilization of the glove in question. The Diacks employed in this study, however, did not melt at all until a temperature of approximately 121° C. was reached. For this reason they could not be used as indicators when gloves were autoclaved at low pressures. On the other hand they melted almost immediately when the requisite temperature was attained, and therefore, theoretically at least, did not necessarily give evidence concerning the time factor involved in the complete sterilization of spores at any given temperature.

It, therefore, seemed desirable to further assay the value of this indicator, and a new type of experiment was devised. Two gloves were contaminated with *Cl. oedematiens* and a Diack and a Sterilometer were tied together and

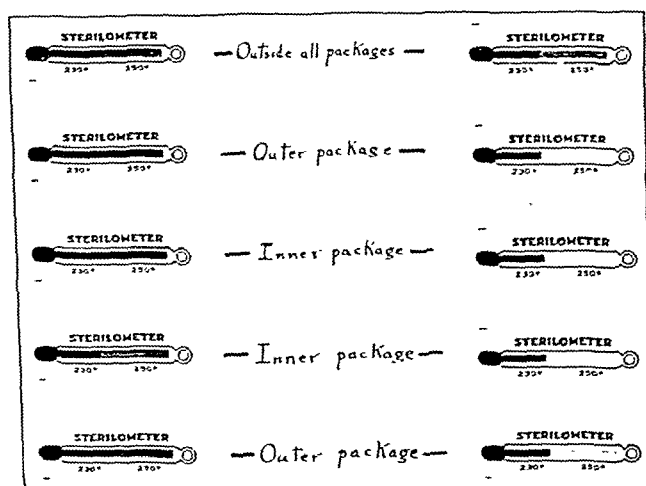


Fig. 1.

placed in the cuff of each of them. The gloves were wrapped in a small package containing only one glove book. They were autoclaved at a high pressure for a short time, following which cultures were made in the manner previously indicated. A number of these experiments were performed, the times in the autoclave and the pressures attained being varied. The protocol of one such experiment is here given:

Total time in autoclave	3½ minutes
Highest pressure attained	19 pounds
Diack	melted
Sterilometer	incompletely darkened
Culture from glove	growth

Results such as this were obtained on two occasions. In these tests the efficacy of the Sterilometer was again upheld, whereas it was shown that it is possible to demonstrate instances in which the melting of a Diack will not serve as an indicator of complete sterility.

The importance of the penetration of steam in sterilization is also illustrated by the following results: In 34 instances of cultures showing growth, a record was kept of whether the swabs, from which the cultures were made, had been taken from an inside or an outside package. Of these, 7 swabs were taken from outside packages, whereas 27 came from inside ones.

DISCUSSION

It is of interest to compare the results of the experiments reported above with previous work on the heat resistance of various spores. Bigelow and Esty⁶ reported that some thermophilic organisms would withstand considerably more heat for a given time than did any of the spores employed in our work. It is obvious that the sterilization indicators used by us would probably have been inadequate as an index of sterility if the gloves had been contaminated with spores of these thermophiles. These organisms, however, do not appear to be of any consequence as a surgical problem.

The spores of *Cl. parabotulinum A* are generally considered to be more heat-resistant than are those of any other organism pathogenic to man. Meyer,³ in 1931, quoting the work of Esty and Meyer⁷ in 1922, reports the following figures as indicating the "maximum heat resistance of *Cl. parabotulinum* spores produced in a pea peptic digest medium under optimum conditions of growth:

"33 to 36 minutes at 110° C. or 6.1 pounds
11 to 12 minutes at 115° C. or 9.8 pounds
2 to 8 minutes at 120° C. or 14.1 pounds"

Although this organism can hardly be said to be of surgical significance we decided to check the Sterilometer against these maximum survival times. These indicators were therefore sterilized free in the autoclave at the longest time intervals recorded in the above table and at the corresponding pressures. None of these Sterilometers were completely blackened. It therefore appears safe to assume that a completely blackened Sterilometer would indicate complete sterilization of a glove even though it had been contaminated with an organism as resistant as the above strains of *Cl. parabotulinum A*.

Most of the organisms employed by us may be said to be of great surgical significance. Fortunately their heat resistance appears to be relatively low, when compared with that of the above bacteria. Esty and Meyer⁷ reported that the heat resistance of a number of the anaerobes associated with gas gangrene was markedly less than that of *Cl. parabotulinum A*. Murray and Headlee⁸ found that spores of *Cl. tetani* would be effectively destroyed in physiologic saline at the following times and temperatures:

25 to 60 minutes at 95° C.
10 to 25 minutes at 100° C.
5 to 10 minutes at 105° C.

Murray⁹ found the following times and temperatures effective for the destruction of anthrax spores under like conditions:

15 to 45 minutes at 90° C.
10 to 25 minutes at 95° C.
5 to 10 minutes at 100° and 105° C.

Finally Headlee¹⁰ reported that spores of *Cl. welchii* would be destroyed under like conditions in

35 minutes or less at 90° C.
15 minutes or less at 95° C.
10 minutes or less at 100° C.

From the work reported it seems that a completely blackened Sterilometer may, when properly used and interpreted, be regarded as an indicator of the adequate sterility of rubber gloves. This is especially the case inasmuch as this indicator has been successfully checked against more than 400 cultures of *Cl. oedematiens*, which appears to be a highly resistant organism. The penetration of steam has been shown to vary considerably when glove packages are packed closely together. For this reason it is essential that the individual glove packages be carefully arranged in the autoclave to permit the maximum access of steam to each. We feel that gloves may be safely sterilized at 10 pounds for thirty minutes, 15 pounds for twenty minutes, or 20 pounds for fifteen minutes, provided that the autoclave is functioning properly and that the penetration of steam is sufficient. A suitable indicator, if placed in the center of each glove package, should act as an adequate check as to the efficacy of the sterilization obtained under the above conditions.

SUMMARY

Rubber gloves were contaminated with different spore-forming organisms, wrapped in a uniform fashion, and autoclaved at varying times and pressures. They were then tested for sterility.

Minimum sterilizing times for gloves, autoclaved at varying pressures under the experimental conditions employed, were determined.

Cl. oedematiens was found to be the most heat-resistant of the organisms used.

The values of two indicators of sterility, the Diack and the Sterilometer, were checked in connection with these glove sterilization experiments.

The Sterilometer, when properly used and interpreted, was found to be an adequate indicator of the sterility of the gloves in question.

Gloves may safely be sterilized at 10 pounds for thirty minutes, 15 pounds for twenty minutes, or 20 pounds for fifteen minutes, provided that the autoclave is functioning properly and that adequate penetration of steam has occurred.

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VARIABILITY IN THE CORN COMPONENT OF A RACHITOGENIC DIET

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IN A PREVIOUS communication¹ we recommend that before yellow corn (maize kernels) be used in a rachitogenic diet, it should be stored for at least six months after grinding. We have found that this procedure reduces variations in the intensity of rickets and increases the severity of the rickets produced in rats on the Steenbock diet No. 2965. Bourdillon and coworkers² have recently substantiated this finding.

Further work in these laboratories supports our original findings and confirms our opinion that the content of antirachitic substance in corn is responsible for the variations. This work consists of testing forty samples of yellow corn obtained during June and July, 1931, from nineteen different states of the United States. Five pounds of each sample were finely ground and stored for six months at room temperature in white, soda-glass, cork-stoppered bottles. An equal portion of each sample was stored under the same conditions but ground at the end of the storage period rather than at the beginning. After storage, a sample of each corn, stored after grinding and ground after storing, was incorporated as the sole corn component of a batch of the Steenbock ration.

These eighty rations, differing only in respect to their corn component, each prepared with the same care to secure uniformity in compounding, were fed for the usual twenty-one days to Wistar strain albino rats, of average initial weight 50 to 55 gm. in groups of four each. Every animal was weighed each second day throughout the test.

The degree of rickets was determined by the "line test" and confirmed by x-ray. The results are expressed as +, ++, +++, +++++, in increasing order of severity. The average degree of rickets for each group is displayed in Table I and in Fig. 1.

Chemical determinations on the forty corn samples included moisture, ash, fat (petroleum ether thirty-hour Soxhlet extract), calcium (modified method of Neumann³ and of Treadwell and Hall,⁴ and phosphorus (modified methods of Neumann³ and Johnson⁵). Condensed results are given in Table I. It may well

¹From the Department of Biology Research Laboratories and Public Health, Massachusetts Institute of Technology.

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be that there are phosphorous linkages which vary in assimilation properties. We are analyzing for these various phosphorus fractions and will report on them later.

The most significant chemical constituents, because of the known importance of the Ca/P ratio in this diet, are calcium and phosphorus. In our analyses, the size of the sample taken (10 gm.) proved insufficient to give a quantitation of calcium. Check determinations made subsequently upon larger samples by us, and the subsequent oral presentation of corroboratory work by Holmes,⁶ showing that the expectancy for calcium in corn is of the order of about 0.05 per cent, make it obvious that in this ration, in view of its large proportion of added

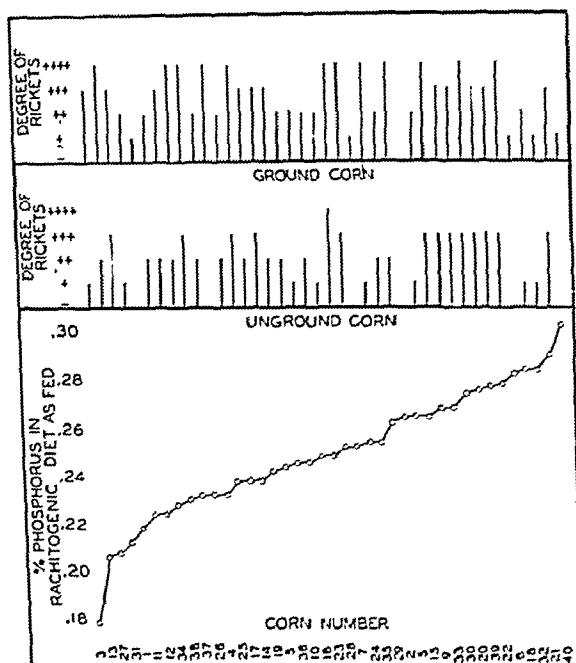


Fig. 1.

CaCO_3 , the calcium constituent of the corn itself has an insignificant effect upon the total Ca/P ratio of the diet as compounded.

Phosphorus determinations showed that there is considerable variation in the amount of this element in the different samples of corn. This variation was so large that the Ca/P ratio of the diet compounded with sample No. 40 was 3.98/1, and 6.7/1 with sample No. 3. The degree of rickets produced by these two samples, when used freshly ground, was "none" and "one +," but when stored after grinding it was "two -" and "three -" respectively. While actual determinations of phosphorus were not made upon the same corn sample both before and after storage, there seems no valid reason for a decrease in total phosphorus (on a dry basis) during storage, and it is therefore assumed that the total phosphorous content of the stored ground corn represents the content of a similar sample stored in the whole kernel. An examination of Fig. 1 indicates that neither the total phosphorus nor the Ca/P ratio of the

TABLE I

CORN NO.	BREED AND SOURCE	MOISTURE %	DRY WT. BASIS			IN DIET AS USED: P %	AV. RAT WT. INCREASE IN 21 DAYS IN GM.		DEGREE OF HICKETS		
			ASH %	PET. ETHER SOL. SUB. %	CA %		P %	CORN		CORN	CORN
								UNGROUND	GROUND		
1	Haskins yellow, Mass.	8.4	1.3	4.2	—	0.312	14	10	none	slight	
2	Golden glow dent, Mass.	8.2	1.6	4.5	—	0.382	28	19	none	none	
3	Lancaster sure crop, N. J.	12.1	1.5	3.9	—	0.268	18	11	slight	moderate	
4	Clarage, Ohio	8.4	1.4	4.6	—	0.332	12	10	fair	fair	
5	Lancaster sure crop, Del.	4.0	1.4	3.7	—	0.336	14	13	fair	fair	
6	Minn. 13 (Sd 86), S. Dak.	8.0	1.6	4.6	—	0.280	18	13	none	fair	
7	Williams yellow dent, Mass.	8.3	1.6	4.3	—	0.364	20	8	none	fair	
8	Minn. 13 Thorpe, N. D.	11.5	1.4	3.6	—	0.396	14	9	slight	fair	
9	Duncan yellow dent, Mich.	11.4	1.5	4.8	—	0.400	17	19	moderate	moderate	
10	Not known, Wis.	9.0	1.6	4.6	—	0.359	16	14	fair	fair	
11	Thatcher's gold dent, Ark.	7.9	1.4	3.7	—	0.320	8	12	fair	fair	
12	Not known, Ill.	13.7	1.2	3.9	—	0.340	9	9	fair	fair	
13	Longfellow-Davis flint, Mass.	9.3	1.3	4.3	—	0.300	10	12	fair	severe	
14	Not known, Ill.	12.4	1.9	4.3	—	0.358	18	16	moderate	moderate	
15	Tulsart yellow dent, N. J.	13.3	1.3	4.1	—	0.404	17	14	moderate	severe	
16	Kruger, Ill.	12.3	1.4	4.3	—	0.372	14	6	slight	fair	
17	Golden glow, Mich.	11.6	1.5	4.1	—	0.354	15	15	fair	moderate	
18	Yellow dent seed corn, Utah	12.2	1.5	4.2	—	0.427	13	13	slight	fair	
19	Sure crop, Pa.	8.0	1.3	4.4	—	0.345	13	11	fair	moderate	
20	Hall's gold nugget flint, N. J.	12.7	1.5	4.6	—	0.416	8	6	moderate	moderate	
21	Polar dent, Mich.	11.6	1.5	4.7	—	0.432	8	8	moderate	moderate	

TABLE I—CONT'D

CORN NO.	BREED AND SOURCE	MOISTURE %	DRY WT. BASIS			IN DIET AS USED: P %	AV. RAT WT. INCREASE IN 21 DAYS IN GM.		DEGREE OF RICKETS	
			ASH %	PER. ETHER SOL. SUB. %	CA %	P %	UNGROUND	CORN	UNGROUND	CORN
22	St. Paul 13 dent, Minn.	12.0	1.6	4.0	—	0.414	17	18	moderate	severe
23	MAC yellow dent, Mich.	11.8	1.7	4.4	—	0.370	14	14	severe	severe
24	Reid's yellow dent, Neb.	13.8	1.3	3.6	—	0.389	21	17	slight	severe
25	Golden queen, Pa.	8.2	1.5	4.4	—	0.340	15	13	moderate	severe
26	Western Ploverman, Ill.	12.9	1.2	3.2	—	0.351	18	20	none	severe
27	Reid's yellow dent, Del.	13.6	1.4	4.0	—	0.318	20	13	moderate	moderate
28	Reid's yellow dent, N. J.	12.8	1.5	4.4	—	0.381	23	19	moderate	severe
29	Not known, Kan.	8.4	1.4	4.1	—	0.375	12	4	fair	severe
30	Wilson yellow dent, Fla.	12.2	1.4	4.6	—	0.404	11	12	moderate	severe
31	Somerset teaming dent, N. J.	12.9	1.2	5.0	—	0.320	9	16	slight	fair
32	Cattle, Neb.	14.0	1.5	3.8	—	0.435	16	7	fair	slight
33	King Philip Hybrid, Calif.	12.4	1.0	4.3	—	0.404	15	14	moderate	moderate
34	Pickett yellow dent, Mich.	11.7	1.5	3.8	—	0.340	17	13	fair	severe
35	Reid's white cap, N. J.	12.0	1.5	4.5	—	0.383	10	16	fair	fair
36	Merced flint, N. D.	11.2	1.5	4.4	—	0.374	15	9	slight	fair
37	Merced white cap yellow dent, N. J.	12.2	1.4	4.1	—	0.342	12	13	fair	fair
38	Clement's white cap, Mich.	12.1	1.4	4.1	—	0.341	11	11	moderate	severe
39	White flint, R. I.	12.9	1.9	4.5	—	0.418	13	13	moderate	moderate
40	Moore's white, Wis.	12.5	1.5	4.1	—	0.338	21	19	none	fair
Average		11.4	1.4	4.2	—	0.366	15	13		

finished diet correlates with the variations in degree of rickets produced. In this connection, it may be pointed out that our results do not entirely agree with those reported by Brown and coworkers⁷ in which difficulty in producing rickets upon a ration containing more than 0.25 per cent P is indicated. We heartily agree with their thesis that the percentage phosphorus in rachitogenic diets should be stated.

In view of the evidence herewith presented, we conclude that the presence of a significant amount of antirachitic substance definitely affects the degree of rickets produced and causes wide differences in the rachitogenic properties of the corns tested.

It seems to us a reasonable premise that in testing a substance for its antirachitic potency, the animals at the start of the feeding period should be in a condition caused by the absence of the factor which is to be assayed, not in a condition caused by a partial deficiency of this factor. In testing the ability of a substance to cure rickets, the animals should be rachitic, not partially rachitic. Judged by this criterion, if "freshly ground" corn were used by each of forty laboratories which had been supplied with samples of corn identical with ours, at the end of twenty-one days only one laboratory would have animals with severe rickets, 14 laboratories would have animals with moderate rickets, 13 would have animals with fair rickets, 6 would have animals with slight rickets, and 6 would have failed to produce any rickets at all.

Even if these corns had been ground and then stored before using in the diet, although the results would have been favorable in a larger number of cases (12 samples supporting the production of satisfactory 4+ rickets, 11 giving 3+, 14 causing 2+, 2 producing rickets of 1+ degree, and only 1 failing completely to cause a detectable rachitic condition), still it must be evident that this preliminary grinding and storage for six months is merely a palliative and not a remedy and does not relieve the user of the necessity of preliminary assay of his corn sample.

Space prohibits the discussion of the varying rates of growth with the samples tested and whether this has any bearing upon the results. We can find no correlation between our growth curves and the severity of rickets developed and conclude that the relation is negligible if present at all.

The development of a rachitogenic diet more reliable than those containing variable substances such as corn, is necessary. We have inaugurated experiments toward this end.

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THE RELATION OF BLOOD GLUTATHIONE TO THE HEMOGLOBIN AND AMOUNT OF RED CELLS*

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GLUTATHIONE, found widely in plant and animal tissues, is the chief of the sulphhydryl compounds which have the reversible property of accepting and giving up hydrogen. "The autoreduction and oxydation of glutathione depend on the simultaneous presence of catalytic metals and an additional factor" (Wurmser), conditions found only in living cells. This faculty constitutes an intracellular respiration. In the blood, glutathione exists only in the cells and might, therefore, be expected to follow their fluctuations. We find, however, that it does not do this, that the amount of glutathione even in severe anemia is usually near the normal level, so that its quantity relative to the bulk of the containing cells is increased. The glutathione is, therefore, independent of the hemoglobin, and is rather connected with the metabolism of the erythrocyte as a living cell. Our work suggests that glutathione is larger in amount proportionately in young cells and a normal glutathione in an anemic blood is evidence of replacement.

To learn the relation, if any, of glutathione to hemoglobin, to number of red cells, cell volume, and to the other reducing substances of the blood, we chose for study a series of anemic bloods. In pathologic bloods the wide deviations of the various findings would throw into light immediately any quantitative connection between any group of the constituents, while with the normal of a biologic substance the narrower limits of normal deviation might place some of the elements examined in a specious relationship expressible by a simple figure and made plausible by statistical correlation. While we were working, there appeared the paper of Woodward and Fry¹ on blood glutathione (GSH) in which they computed Gabbe's quotient, $\frac{\text{GSH}}{\text{R.B.C.}}$, and added one of their own, $\frac{\text{GSH} \times \text{Hb}}{\text{R.B.C.}}$. In 23 bloods Gabbe's quotient ranged from 4.9 to 9.1, the quotient of Woodward and Fry from 5.3 to 8.2. This would hardly prove any relation between these elements in normal blood. Our tables of anemic bloods show still more clearly this lack of quantitative relation.

Glutathione† was determined by direct titration with N 1/1,000 iodine using starch as internal indicator, as advised by Delaville and Kowarski,² adding 2 c.c. of 25 per cent KI to 10 c.c. of tungstate filtrate as suggested by Perlzweig and Delrue.³ The iodine solution was checked by titration of a

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standard glutathione solution. We used the ordinary 1/10 tungstate blood filtrate. The stability of glutathione in this filtrate will appear in the note on tissue filtrates.

The hemoglobin was determined by the Newcomer method (B. & L.), a method that is practical and is as accurate as the Van Slyke oxygen capacity method when the Newcomer glass plate has been standardized by the latter,⁴ and with which duplicate tests check so closely as to be unnecessary. As a percentage we report the hemoglobin by Williamson's normal of 16.92 gm. per 100 c.c. for men, 15.53 gm. for women. This seems to be accurate for our locality, San Diego. The careful work of Osgood and Haskins has shown their normal of 15.8 gm. for men, and 13.7 for women to be correct for Oregon where their work was done, but use of their normals made the color and volume indices too high for the clinical picture in our cases. The wide variation of the red cell count and of the hemoglobin in health makes any standard quite arbitrary. The difference between men and women's blood is really a difference perhaps occasioned by the difference between their muscular activity. Sedentary persons should probably be appraised by the normal female standard.

As volume index, $VI = \frac{\text{per cent normal cell volume}}{\text{per cent normal cell count}}$, we took 5 million as normal red cell count, and 48 per cent (Sanford⁵) as normal volume of packed cells. We tried out the volume index by the method of Osgood, Haskins and Trotman,⁶ $VI = \frac{\text{per cent cell volume}}{\text{per cent R. B. C.}}$, when the volume of packed red cells per 100 c.c. blood, calculated to a red cell count of 5 million (men 41 per cent, women 43 per cent packed cells) is taken as 100 per cent cell volume for the sex, and when 5 million per c.mm. is taken as 100 per cent red cells, but this index was considerably too high for our locality, giving with the bloods of the secondary anemias in Table I an average of 1.1; with the anemias from hemorrhage, Table II an average of 0.9; with the subnormal bloods in Table IV, the average was 1.1.

The color index was calculated by dividing the percentage of 5 million cells into the percentage of hemoglobin, obtained as above. So many indices have been advised for clinical blood work that it seemed best to choose these two which are better known and simpler. Both are subject to inexplicable variations. The rule is given that if either the color or volume index is over 1.25, the anemia is pernicious. There are many exceptions to this among our cases.

The apparent blood sugar determined by the Folin-Wu method, as is well known, includes a nonglucose sugar in small amount, the glucid X of Fonte's and Thivolle, saccharoid of Benedict, which is not glutathione.⁷ The nonglucose reduction beyond that of the Folin-Wu figure, as measured by the Ionesco ferrieyanide technic⁸ in the same tungstate filtrate is reported in terms of glucose. This, the Y-reduction first reported by Pickard, Pierce, Marsden, Tanaka and Townsend,⁹ includes the reduction given by glutathione. One-half the figure for the amount of glutathione in milligrams per 100 c.c. subtracted from the Y-reduction (excess of the Ionesco technic over the Folin-Wu,

as glucose) leaves the variable part of the Y-reduction, due to unknown substances which are greatly increased in certain pathologic conditions. Glutathione is separated from the other part of the Y-reduction by the preparation of unlaked blood filtrates (Folin), it being then precipitated with the red cells.¹⁰

The bloods are arranged in the tables in order of increasing amounts of glutathione. Table I gives the findings in 23 cases of secondary anemia of

TABLE I
NONHEMORRHAGIC SECONDARY ANEMIAS

PATIENTS REFERRED TO BY NUMBER TOGETHER WITH NAME OF ATTENDING PHYSICIAN		RED CELL COUNT IN MILLIONS	HEMOGLOBIN, GRAMS PER 100 C.C. NEWCOMER HEMOGLOBINOMETER, H. & L.	GLUTATHIONE IN MG. PER 100 C.C.	CELL VOLUME, ACTUAL PERCENTAGE OF CORPUSCLES, WINTROBE HEMATOCRT.	HEMOGLOBIN IN PERCENTAGE, WILLIAMSON'S NORMALS, 16.92 MALE, 15.53 FEMALE	COLOR INDEX	VOLUME INDEX, OSGOOD	BLOOD SUGAR, FOLIN-WU, MG. PER 100 C.C.	REDUCTION, JONESCO TECHNIC, AS GLUCOSE, MG. PER 100 C.C.	Y-REDUCTION, EXCESS OF JONESCO OVER FOLIN-WU, MG. PER 100 C.C., AS GLUCOSE
1. Oatman	Secondary anemia	4.1	14.5	6.6	40	m86	1.1	1.0	82	102	20
2. Oatman	Diabetes	4.4	13.8	8.8	41	m82	0.9	1.0	145	158	13
3. Ramer	Hodgkin's disease	4.0	11.0	11.5	49	f71	0.9	1.2	124	180	56
4. Molitor	Hypertrophy prostate	3.2	10.8	15.0	-	m64	1.0	-	78	170	92
23. Marsden, Sr.	Abscessed teeth	4.8	12.4	17.4	37	f80	0.8	0.8	94	112	18
5. Doria	Postinfluenza	4.0	12.0	20.0	31	m71	0.9	0.8	85	164	65
6. H. Newton	Aplastic	3.9	14.0	20.0	44	m87	1.1	1.2	107	123	16
7. Ball	Infection	-	9.0	20.0	38	m54	-	-	128	152	24
8. Kennell	Aplastic secondary	3.8	12.2	21.6	35	f78	1.0	1.0	88	126	38
9. Chartres	Typhoid	4.3	8.3	24.1	40	f55	0.6	1.0	180*	241	61
10. Alberty	Neurosis	4.2	15.0	24.6	45	m89	1.0	1.1	93	124	31
11. Ratty	Diabetes	4.2	12.8	25.0	42	f82	1.0	1.0	145	200	55
12. Miller	Diabetes	3.2	9.6	26.5	35	m57	0.9	1.1	260	380	120
13. Hileman	Optic atrophy	4.4	11.2	27.0	40	f72	0.8	0.9	85	162	77
14. Ball	Hyperthyroidism	2.9	5.4	28.0	22	f35	0.6	0.8	-	-	-
15. Eager	Infection	3.9	12.3	28.3	29	f79	1.0	0.8	105	190	85
16. Molitor	No. 4, 3 weeks later	4.6	11.8	29.2	32	m70	0.8	0.7	95	145	50
17. Molitor	No. 4, 2nd week, operated	3.1	13.0	33.0	29	m77	1.2	1.1	101	276	175
18. Kennell	Sinusitis	3.5	12.8	34.6	41	f82	1.2	1.2	110	160	50
19. Fox	Diabetes	3.8	12.8	35.4	34	m76	1.0	0.9	87	117	30
20. Kerch	Postinfluenza	5.1	13.2	37.2	41	m78	0.8	0.8	92	136	44
21. Worthington	Neurosis	4.0	13.5	39.2	40	m80	1.0	1.0	117	204	81
22. Potter	Aplastic secondary	4.0	12.2	40.1	31	f78	0.9	0.8	100	150	50
Averages		3.9	11.9	24.9	37	73%	0.9	1.0			

*After intravenous glucose.

either hemolytic or aplastic type. The glutathione is normal except in four cases and in these bloods the anemia is not particularly marked, but in all four the diseased condition is of several years' duration. Table II lists 10 cases of anemia from hemorrhage. The patient in Case 31 had a glutathione of 4.2 mg.; a week later (blood 40), with a rise in the red cells from 1.6 to 2.5 millions, the gluta-

thione was normal, 30 mg. per 100 c.c. Assuming 30 mg. glutathione and 5 million cells as normal, the concentration of glutathione in samples 31 and 40 is respectively 50 per cent and 200 per cent of the normal concentration. Calculated to a normal cell volume of 46 per cent the glutathione concentration is 64 per cent (31) and 250 per cent (40). In the erythrocytes put in circulation immediately after hemorrhage, the glutathione was exceptionally low. Its restoration to normal in a week would seem indicative of its importance. The same drop after hemorrhage occurred in Case 51 (Table VI). Unfortunately we were unable to obtain follow-up specimens on other patients in Table II. In these

TABLE II
POSTHEMORRHAGIC ANEMIAS

PATIENTS REFERRED TO BY NUMBER TOGETHER WITH NAME OF ATTENDING PHYSICIAN		RED CELL COUNT IN MILLIONS	HEMOGLOBIN, GRAMS PER 100 C.C. NEWCOMER HEMOGLOBINOMETER, B. & L.	GLUTATHIONE IN MG. PER 100 C.C.	CELL VOLUME, ACTUAL PERCENTAGE OF CORPUSCLES, WINTROBE HEMATOCRIT.	HEMOGLOBIN IN PERCENTAGE, WILLIAMSON'S NORMALS, 16.92 MALE, 15.53 FEMALE	COLOR INDEX	VOLUME INDEX, OSGOOD	BLOOD SUGAR, FOLIN-WU, MG. PER 100 C.C.	REDUCTION, IONESCO TECHNIC, AS GLUCOSE, MG. PER 100 C.C.	Y-REDUCTION, EXCESS OF IONESCO OVER FOLIN-WU, MG. PER 100 C.C., AS GLUCOSE
31. Ball	Abortion	1.6	4.1	4.2	10	f26	0.8	0.7	89	155	61
32. Ball	Intestinal hemorrhage	0.7	2.3	9.0	6	m16	1.4	0.9	160*	174	14
33. Ball	Obstetric	2.2	5.8	11.5	32	f37	0.8	1.5	87	126	39
34. Ball	Obstetric	1.9	5.5	11.6	12	f35	0.9	0.7	-	72	-
35. Ball	Abortion	3.3	7.5	12.5	20	f48	0.7	0.6	118	154	36
36. Ball	Duodenal ulcer	2.4	7.3	13.0	20	m43	0.9	0.9	87	119	32
37. Ball	Carcinoma of sigmoid	1.8	5.5	21.2	7	f53	0.9	0.4	95	112	17
38. Ball	Obstetric	2.5	6.5	21.8	21	f42	0.8	0.9	70	105	35
39. Crawford	Hemorrhage of intestine	1.9	5.9	21.9	15	f38	1.0	0.8	110	160	50
40. Ball	31, week later	2.5	6.2	30.0	18	f40	0.8	0.8	90	120	30
41. Oatman	Hemorrhage fibroid	4.2	10.2	31.5	32	f66	1.0	0.8	118	-	-
Averages		2.3	6.1	17.1	17.5	40	0.9	0.8			

*After intravenous glucose.

bloods of anemia from hemorrhage the glutathione follows somewhat the loss in cells and hemoglobin. In nearly all the bloods in Table I, the glutathione is in the normal range of 20 to 40 mg., average 30 mg., and its importance to the metabolism of the erythrocyte can be deduced from this together with the absence of any ratio between the amount of glutathione and the number of cells, amount of hemoglobin, cell volume percentage, or the amount of the other reducing bodies. Graphs drawn experimentally to trace any trend or grouping of the GSH and other blood findings showed only the widest divergence. We believe that the tabulated results bear out the idea that the hemoglobin and cell volume percentage should be reported in their actual amounts,

as are the red cell count and the blood chemical findings, rather than as a percentage of normal averages, which latter, like the indices, can be misleading. The clinician should base his diagnosis on findings referred to the normal range for the individual type. Ratios between the findings not apparent on inspection are apt to be misleading or unimportant.

Table III gives the glutathione in 7 cases of anemia diagnosed as pernicious; this is not enough from which to draw any conclusions but the low glutathione is noticeable. The bloods in Table IV are mostly in the lower limit of normal but were placed there because of apparent good health. None of the eight were doing any regular active muscular work. Except the patient in

TABLE III
PERNICIOUS ANEMIAS

PATIENTS REFERRED TO BY NUMBER TOGETHER WITH NAME OF ATTENDING PHYSICIAN		RED CELL COUNT IN MILLIONS	HEMOGLOBIN, GRAMS PER 100 C.C. NEWCOMER HEMOGLOBINOMETER, R. & L.	GLUTATHIONE IN MG. PER 100 C.C.	CELL VOLUME, ACTUAL PERCENTAGE OF CORPUSCLES, WINTROBE HEMATOCRIT.	HEMOGLOBIN IN PERCENTAGE, WILLIAMSON'S NORMALS, 16.92 MALE, 15.53 FEMALE	COLOR INDEX	VOLUME INDEX, OSGOOD	BLOOD SUGAR, FOLIN-WU, MG. PER 100 C.C.	REDUCTION, IONESCO TECHNIC, AS GLUCOSE, MG. PER 100 C.C.	V-REDUCTION, EXCESS OF IONESCO OVER FOLIN-WU, MG. PER 100 C.C., AS GLUCOSE
45. Ball	Pernicious anemia 7 years	0.6	2.0	1.7	6	f12	1.0	1.0	145	156	11
46. Austin	Treated case	-	11.2	11.7	33	-	-	-	-	-	-
47. Alberty	Occasionally treated	3.2	12.5	13.2	30	m74	1.2	1.2	122	146	24
48. Doria	Pernicious anemia 8 years	2.6	12.0	18.1	34	m71	1.2	1.4	100	105	5
50. Ball	Treated	1.0	5.6	27.7	9	f37	1.8	0.9	89	130	41
51. Marsden	Untreated	3.2	12.5	28.6	41	f82	1.2	1.3	85	191	106
52. Roberts	Untreated	3.3	13.0	-	43	f84	1.3	1.3	80	-	-
52. Month later	Treatment	4.4	12.2	34.2	35	78	0.9	0.8	81	128	47

Case 61 all were regular users of alcohol, the distinction of 62 and 64 as "alcoholic" was because they were exceptional in the amount consumed daily, the patient in Case 64 using 300 c.c. (as absolute alcohol) daily, and it was thought that this might influence the blood sugar, in both cases at the upper normal limit.

We were able to follow two cases. Case I was a young man, a college student in poor health in that he easily became exhausted. Physical examination was negative except for a basal metabolic rate of minus 16. His father died of Hodgkin's disease. While his blood smears were negative, the high color and volume indices suggest pernicious anemia. The blood glutathione remained low (Table V). There is evidently a severe metabolic disturbance.

The low basal rate may be due to the low glutathione and resultant lower intracellular metabolism and not to the thyroid.

Case 51, a woman in midlife, was in a run-down condition, asthenic, nervous, worried and suffering from insomnia at the time of the first examination when the Y-reduction was high, 106 mg. The high blood indices suggested pernicious anemia; there were no nucleated cells. The second examination was like the first except that the patient was feeling stronger and sleeping well and the Y-reduction was then normal, 13 mg., of which glutathione accounted for 12 mg. The high red count with lower hemoglobin on December 6 (Table VI) was due to severe menstrual loss; she had been on liver and iron treatment for a week. The glutathione fell to 8.7 mg. with the hemorrhage, and the color index from 1.4 to 0.9.

TABLE IV
NORMAL

PATIENTS REFERRED TO BY NUMBER										
	RED CELL COUNT IN MILLIONS	HEMOGLOBIN, GRAMS PER 100 C.C. NEWCOMER HEMOGLOBINOMETER, R. & L.	GLUTATHIONE IN MG. PER 100 C.C.	CELL VOLUME, ACTUAL PERCENTAGE OF CORPUSCLES, WINTROBE HEMATOCRT.	HEMOGLOBIN IN PERCENTAGE, WILLIAMSON'S NORMALS, 16.92 MALE, 15.53 FEMALE	COLOR INDEX	VOLUME INDEX, OSGOOD	BLOOD SUGAR, FOLIN-WU, MG. PER 100 C.C.	REDUCTION, IONESCO TECHNIC, AS GLUCOSE, MG. PER 100 C.C.	Y-REDUCTION, EXCESS OF IONESCO OVER FOLIN-WU, MG. PER 100 C.C., AS GLUCOSE
61. Age over 60	4.6	12.0	12.3	38	478	0.9	0.9	124	134	10
62. Alcoholic	4.7	15.0	18.4	44	m89	1.0	1.0	115	138	23
63. Aged 50	5.4	16.4	18.4	45	m98	0.9	0.9	112	152	40
64. Aged 50, alcoholic	4.5	14.0	22.0	41	m83	0.9	1.0	120	147	27
65. Student	4.1	16.2	24.6	45	m96	1.2	1.1	85	108	23
66. Student	4.7	15.4	28.3	41	m92	1.0	0.9	95	109	14
67. Student	5.6	15.5	31.7	46	m92	0.8	0.9	101	132	31
68. Sedentary	5.1	16.0	37.5	41	m94	0.9	0.8	81	112	31
Averages	4.8	15.1	24.1	43	91	0.9	0.9			25

The Y-reduction was originally calculated as a difference in reduction between different filtrates and reduction technics, and was then found to average about 40 mg. per 100 c.c. in normal blood.⁹ There have not been many normals examined since the use of the Ionesco technic as a comparison with the Folin-Wu in the same tungstate filtrate. As Fabre¹¹ says, the difficulty of dissolving the sulphydryl derivatives tends to demonstrate that reduced glutathione does not exist in a free state, but in a potential form in the cells of the organism. This is borne out in that copper solutions are not reduced by the glutathione in the blood, while glutathione added to blood before precipitation gives an added reduction with the Folin-Wu (20 per cent as glucose) and Ionesco technics (50 per cent, as glucose) and is also quantitatively recovered

by the iodine titration and in the increase in nonprotein nitrogen.¹⁰ The Ionesco technic shows the reduction of true glucose and of glucid X, as does the Folin-Wu, and in addition the reduction of the glutathione of the cells together with a reduction due to unknown substances.¹⁰ We believe from the present work that the normal fasting Y-reduction nearly approximates that part of the Y-reduction due to glutathione. Certainly a Y-reduction of over 25 mg. shows an increase in the part due to the unknown substances which are increased, or retained, especially in kidney disease.

The possibility that the quantity of glutathione in a tissue might serve as an index to its metabolic activity is suggested by the work of Blanchetière

TABLE V
FOLLOW-UP DATA ON TWO CASES

PATIENTS REFERRED TO BY NUMBER										
	RED CELL COUNT IN MILLIONS	HEMOGLOBIN, GRAMS PER 100 C.C. NEWCOMER HEMOGLOBINOMETER, B. & L.	GLUTATHIONE IN MG. PER 100 C.C.	CELL VOLUME, ACTUAL PERCENTAGE OF CORPUSCLES, WINTROBE HEMATOCYT.	HEMOGLOBIN IN PERCENTAGE, WILLIAMSON'S NORMALS, 16.92 MALE, 15.53 FEMALE	COLOR INDEX	VOLUME INDEX, OSGOOD	BLOOD SUGAR, FOLIN-WU, MG. PER 100 C.C.	REDUCTION, IONESCO TECHNIC, AS GLUCOSE, MG. PER 100 C.C.	Y-REDUCTION, EXCESS OF IONESCO OVER FOLIN-WU, MG. PER 100 C.C., AS GLUCOSE
Case 1. Nov. 16	4.1	14.5	6.6	40	m86	1.0	1.0	82	102	20
	4.3	12.8	7.3	43	76	0.8	1.0	80	92	12
	Jan. 5	3.7	12.5	7.0	40	74	1.6	1.1	100	110
	Feb. 3	4.5	12.8	5.2	76	0.7	-	-	-	-
Case 51. Oct. 28	3.2	12.5	28.6	41	f82	1.2	1.3	85	191	106
	Nov. 7	3.0	12.8	23.8	42	83	1.4	1.5	89	102
	Dec. 6*	4.0	11.5	8.7	42	75	0.9	1.1	100	114
	Jan. 5	4.0	12.5	25.4	43	81	1.5	1.1	110	136

*During severe menstrual hemorrhage.

and Mélon, cited by Wurmser,¹² using dog and rabbit tissues. Mme. Boucher-Firly found that the total amount of glutathione in eels diminished as they grew older, and there is a parallelism between the amount of glutathione and the oxygen consumption.¹³ Murray found the same progressive diminution in glutathione in chicken embryo. His work is cited by M. Labbé, H. Labbé and Nepveux in a résumé of the work done on the clinical aspect of the glutathione content of blood and other tissues.¹⁴ They state that neither fasting nor diet change the glutathione in the blood nor is there any connection between the quantity of glutathione and changes in the basal metabolism. They think that "the mechanism of the action of glutathione is extremely complex, that it is probable that the sulphydryl bodies act at the level of certain stages of the degradation of acid chains where there is no immediate or simultaneous

effect on the terminal stages such as glucose, urea or uric acid." They found a low blood glutathione in many patients with disturbed nutrition: gout, diabetes, cirrhosis, obesity, half or less of the 21 mg. per cent which they find in normal blood, following the technic of Tunncliffe modified by Randoïn and Fabre.

That the comparison with the normal glutathione of the tissue may be an index to the restorative capacity in pathologic conditions is suggested by our tables of anemic bloods. Table VI gives the glutathione content in cavy and human tissues, including three cancers. We macerated the tissues in distilled water for an hour, then precipitated with tungstic acid to obtain a 1/10 filtrate as with blood. There has been some criticism of the tungstate extraction of glutathione. Following Tunncliffe most of the work with glutathione has been done on triochloroacetic acid filtrates. Repeated tests on our tissue filtrates either alone or standing on the tissue and continuing the ex-

TABLE VI
GLUTATHIONE IN TISSUES (FIGURES, MILLIGRAMS PER 100 GM.)

1. Guinea pig—muscle	36.9	5. Human—papillomas from ovarian cyst	145.0
2. Guinea pig—muscle	54.6	6. Human—cancer breast mass under skin	220.0
liver	397.0	deep in breast	336.0
(pregnant) uterus	170.0	same mass deep in breast	320.0
placenta	81.6	pectoral muscle	131.0
3. Guinea pig—muscle	50.7	same muscle	127.0
same, after 2 weeks' extraction	50.6	fat	0.9
4. Guinea pig embryo—muscle	54.6	7. Human—cancer breast, recurrent	140.8
liver	170.0	8. Human—fibrous mastitis	1.9
		9. Human—uterine muscle	125.9
		decidua	45.5
		10. Human—embryonal cancer testis	475.0

traction, checked so closely after hours and even days that we give the result of one test in which the extraction continued two weeks and the glutathione determination was the same within the limits of error. The tungstate extraction seems to be immediate and complete. For these reasons we do not think the criticism of tungstate filtrates for glutathione are valid. We tried the sulphosalicylic acid filtrate advised by Woodward and Fry but did not get a distinct end-point with the iodate titration, and the results were lower than with tungstate and iodine.

With guinea pig liver and striated muscle our results correspond with those obtained by Blanchetière and Mélon. In the embryo cavy the liver is a tremendous storehouse for glycogen, nearly 2.8 per cent as glucose. The fetal liver has a low glutathione content compared to that of the adult liver when the digestive function is active. The glutathione in the pregnant uterine muscle of the cavy was much higher than that in the smooth muscle examined by the French investigators. The highest glutathione we found was in an embryonal carcinoma of the testis, and except for liver the largest amount

found in other tissues was in parts of an actively growing cancer of the breast. This is in accord with the findings of Thompson and Voegtlin (cited by Wurmser). Their statement that these tumors seem to collect glutathione and that it diminishes in the rest of the organism is contradicted by Labbé and Nepveux, and for the blood at least is not sustained by Woodward and Fry, who found a normal blood glutathione in their six cancer cases. In the embryonic carcinoma of the testicle, cancer of the breast, papilloma and other tissues the area in the microscopic picture occupied by nuclei is in direct ratio with the glutathione content of the tissue, suggesting that this substance is chiefly in the nuclear protoplasm. Fat, containing almost no cytoplasm, had the lowest glutathione, almost as low as was given by the dense white fibrous tissue from a chronic mastitis. Tissue activity and the amount of glutathione seem to correlate to such an extent that the latter may be taken as an index to intracellular metabolism.

SUMMARY

The blood glutathione is necessarily lowered when there is a sudden loss of blood, but its amount is early restored to the normal preceding that of the number of red cells and the hemoglobin. There is no quantitative relation between the hemoglobin and the glutathione of the blood. Although the glutathione of the blood is part of the content of the red cells, its quantity does not follow variations in either the cell count or the cell volume. The average amount of glutathione in 2 patients with nonhemorrhagic secondary anemia (Table I) was 24.9 mg.; excluding the 5 bloods with glutathione under 20 mg., the average was 28.5 mg. For the bloods taken after severe hemorrhage (Table II) the average glutathione was 17.1 mg., average red count 2.3 million, average cell volume 17.5 per cent. Calculated to a red count of 5 million, the glutathione would be 37 mg., a 23 per cent excess concentration in the cells, and to a normal cell volume of 46 per cent, there is a 150 per cent glutathione concentration. This gives added emphasis to the findings in Case 1, Table V. In this patient the glutathione as calculated for 5 million cells is 8.0, 8.5, 9.5, 5.8 mg. for the four examinations, averaging only 27 per cent of the normal concentration as to cell count, and 26 per cent of the normal as to cell volume. The persistently low glutathione in the blood of this patient, asthenic, but without definite physical findings, constitutes a deficiency in cell protoplasm new to medical literature.

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THE INACTIVATION OF GROWTH HORMONE*

II. AS A RESULT OF EXPOSURE TO AIR

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IN THE course of experiments dealing with the effect of growth hormone upon the brain-weight-body weight ratio¹ it was noted that in order to maintain continuous body growth in rats (*Mus norvegicus albinus*) it was necessary to use fresh extract almost every other week. This became necessary because after an initial growth stimulating effect, it was noted that the extract soon lost its potency. It therefore became quite an interesting problem to determine the factors causing this loss of potency of the extract and recently this laboratory reported improper refrigeration to be one of these.²

As a result of this, it was found advantageous to place a few drops of phenolphthalein as an indicator into each bottle of growth hormone and to use the extract only as long as the P_H was maintained at approximately 8.3. The addition of phenolphthalein led to a rather interesting observation; namely, that pouring the extract into a beaker prior to administration caused a fading out of the red color with a consequent loss of alkalinity. At first this phenomenon was believed to be due to acid fumes in the air since our laboratory is situated in an industrial center.

In order to rule out such an atmospheric factor, 30 c.c. of growth hormone were placed in an open beaker within a refrigerator whose temperature was kept at 10° C. In another beaker, were placed 30 c.c. of meat extract which is ordinarily used for control injections. The alkalinity of each extract was just sufficient to give a pink tinge to phenolphthalein (P_H 8.3). These were

*From the Neuroanatomical Laboratory of the Department of Anatomy, University of Maryland Medical School.

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then allowed to stand, thus exposed to the cold air of the ice box, for twenty-four hours, at the end of which time, the growth hormone solution was completely decolorized, while the meat extract maintained its color. The only single factor which could possibly have decreased the alkalinity of the growth hormone was air, since refrigeration was adequately maintained. Furthermore, as long as the extract was kept stoppered no diminution in its alkalinity could be noted.

If allowed to stand long enough, this alkalinity will be decreased even to the point of becoming truly acidic, since Morrell³ checking up on some of our findings noted in one case a drop in P_H from 8.3 to 6.76.

The necessity for keeping the solutions well stoppered even during injections becomes quite apparent since, as in the case where inactivation is the result of inadequate refrigeration, growth effects can be obtained only with definitely alkaline material.

CONCLUSIONS

1. Growth hormone is active only so long as it is alkaline.
2. Alkalinity is diminished and even lost when the hormone is exposed to the air.

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LABORATORY METHODS

THE DETERMINATION OF HEMOGLOBIN BY THE IRON CONTENT METHOD*

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THE presence of iron in blood was first demonstrated by Menghini of Bologna in 1747,¹ and for more than a century the study of this problem has excited much interest. Quantitative analyses were made by Denis in 1830,² and quite accurate estimations were accomplished by others before 1850. The early investigators obtained a remarkable uniformity of results³ by working with relatively large amounts of blood.

After the isolation of hemoglobin in crystalline form by Funke in 1851,⁴ analyses of hemoglobin crystals by Hoppe-Seyler⁵ showed sufficient iron in the crystal to account for all the iron found in whole blood. In the first monograph on hemoglobin, published in 1871,⁶ Preyer compared the quantitative determination of hemoglobin by the spectroscopic, iron content, and colorimetric methods and found that they gave identical results. In 1877,⁷ Malassez' first paper on clinical methods for estimating hemoglobin described three chemical methods, by determination of the iron content, of the oxygen capacity, and of the hematin content of the blood. Even at this early date in clinical hemoglobinometry, however, the colorimetric method already was well established and numerous procedures were in use. The iron content method was little used for many years, although the ferrometer described by Jolles in 1898⁸ was employed to some extent and papers on the iron content of the blood appeared from time to time.

In recent years, there has been much improvement in the methods for estimating hemoglobin and renewed interest in the two available accurate chemical methods. This has been due largely to the development by Wong⁹ of a simple and precise microchemical method for the determination of iron and also to Van Slyke's¹⁰ development of a blood gas apparatus in which oxygen capacity of the blood can be determined correctly by using small quantities of blood. Laboratory workers agree that all hemoglobinometers should be standardized by some accurate chemical method, and the oxygen capacity method has so far been the one preferred. Blood gas analyses, however, require experience and special apparatus. The determination of iron is a simpler procedure, and if it yields results as accurate as the measurement of hemoglobin by the oxygen capacity method, it should be of equal value as a method of standardization and

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also should be available for special laboratory estimations of hemoglobin. If the estimations of hemoglobin by the iron content and by the oxygen capacity methods check within the limit of error, it is apparent that the ratio must be a constant one and that the iron content is an accurate measure of the amount of hemoglobin present. For all practical purposes, the iron content of the whole blood may be considered an indicator of the hemoglobin just as the oxygen capacity is taken as a measure of hemoglobin.

The oxygen capacity method determines all the hemoglobin present in blood except the small amount combined with carbon monoxide. Gettler¹¹ has shown

TABLE I

COMPARISON OF HEMOGLOBIN DETERMINATIONS BY OXYGEN CAPACITY AND IRON CONTENT METHODS

OBSERVER	NUMBER OF DETERMINATIONS	METHOD	HEMOGLOBIN		
			IRON CONTENT	OXYGEN CAPACITY	
Lindsay, Rice and Sellinger	7	Original Wong	Gm. per 100 c.c.	Gm. per 100 c.c.	
			7.89	7.88	
			8.13	9.06	
			12.48	12.21	
			14.85	14.15	
			15.87	15.16	
			15.79	15.89	
			18.80	18.78	
			Mean	13.40	13.30
Kennedy	8	Kennedy	12.55	12.45	
			13.20	12.80	
			14.70	14.52	
			17.00	16.90	
			17.42	17.38	
			17.96	17.90	
			19.70	19.60	
			20.10	20.40	
			Mean	16.58	16.49
Haden	10	Dupray modification of original Wong	12.50	12.40	
			12.95	13.28	
			12.98	13.15	
			13.32	13.43	
			13.46	13.13	
			14.63	14.95	
			14.68	15.02	
			14.68	15.11	
			16.25	15.82	
			17.53	17.31	
			Mean	14.30	14.36
Haden	11	New Wong method	6.2	6.0	
			7.8	7.6	
			8.4	8.0	
			9.4	9.4	
			10.3	10.6	
			10.7	10.4	
			11.8	11.2	
			11.8	12.1	
			12.1	12.4	
			14.4	14.4	
			15.4	15.7	
			Mean	10.2	10.2
			Mean of 36 determinations		

that the carbon monoxide content of the blood is greater in city than in country dwellers but is never sufficient to interfere with clinical determinations of hemoglobin. Riecker¹² found a small amount of iron (1.1 ± 0.022 mg.) in the blood plasma of normals. The remainder of the iron present in blood is in the hemoglobin molecule. In a recent article Reich and Tiedeman¹³ have disputed the value of the iron content of the blood as a measure of hemoglobin and have concluded that the ratio between hemoglobin and iron is not constant; they used in their study a colorimetric estimation of hemoglobin.

From time to time over several years I have determined the iron content and the oxygen capacity of specimens of oxalated blood. Lindsay, Rice and Sellinger,¹⁴ and Kennedy¹⁵ have made similar studies. The values for hemoglobin, when calculated in these two ways, have always checked closely and well within the limit of error. I have compiled the data so far available in Table I. In 36 determinations, the mean hemoglobin by the iron content method is 13.33 gm. and by the oxygen capacity method, 13.34 gm. These results indicate that there is no variation in the iron hemoglobin ratio not accounted for by errors in estimation, and prove that the iron content is as accurate a measure of the hemoglobin as is the determination of the oxygen capacity.

My early determinations were made by the Dupray¹⁶ modification of the original Wong method. In this procedure, perchloric acid is added to facilitate digestion and nitric acid to prevent fading of the iron sulphocyanate. Kennedy¹⁵ suggested the digestion of the blood with perchloric and sulphuric acids, and subsequent extraction of the digested material with sodium sulphocyanate and amyl alcohol to prevent fading. In Wong's new method,¹⁷ the iron is split off from the hemoglobin with sulphuric acid and potassium persulphate, and the interfering proteins are precipitated with tungstic acid, and sodium sulphocyanate is added to the filtered solution. The potassium persulphate prevents the fading of the iron sulphocyanate for a long time. I have found no difference in the results obtained by different methods. The new method of Wong¹⁷ is as follows:

REAGENTS REQUIRED

"All the reagents except the standard must be iron-free as shown by blank tests.

"1. Concentrated Sulphuric Acid.

"2. Sodium Tungstate.—Dissolve an appropriate amount of good grade sodium tungstate to make a 10 per cent solution.

"3. Saturated Potassium Persulphate.—Introduce into a small glass-stoppered bottle about 7 gm. of pure potassium persulphate and shake up with 100 c.c. of distilled water. The undissolved portion settles on the bottom to make good any decomposition upon standing.

"4. Potassium Sulphocyanate.—Prepare approximately a 3 N solution by dissolving 146 gm. of pure potassium sulphocyanate in distilled water to make 500 c.c. Filter, if necessary. Add 20 c.c. of pure acetone to improve its keeping quality.

"5. Standard Iron Solution.—Weigh accurately 0.7 gm. of crystallized ferrous ammonium sulphate and dissolve in about 50 c.c. of distilled water. Add to the solution 20 c.c. of dilute (10 per cent) iron-free sulphuric acid, warm slightly, and then add 0.1 N (approximate) potassium permanganate solution to oxidize the ferrous salt completely. Dilute with distilled water to one liter exactly. Each cubic centimeter will contain 0.1 mg. of iron for use as a regular standard. To make weaker standards dilute this standard solution accordingly.

PROCEDURE

"Transfer accurately with an Ostwald pipette 0.5 c.c. of blood into a 50 c.c. volumetric flask and introduce 2 c.c. of iron-free concentrated sulphuric acid. Whirl the flask to agitate the mixture for one or two minutes. Add 2 c.c. of saturated potassium persulphate solution and shake. Dilute to about 25 c.c. with distilled water and add 2 c.c. of 10 per cent sodium tungstate solution. Mix. Cool to room temperature under the tap and then dilute to volume with distilled water. Stopper the flask and invert two or three times to effect thorough mixing. Filter through a dry filter paper into a clean, dry receiving vessel. Pipette exactly 20 c.c. of the clear filtrate into a large test tube graduated at 20 and 25 c.c.

"Measure into another similar test tube exactly 1 c.c. of the standard iron solution containing 0.1 mg. of Fe per c.c. Add with a graduated 1 c.c. pipette 0.8 c.c. of iron-free concentrated sulphuric acid and dilute to the 20 c.c. mark with distilled water. Cool to room temperature under the tap. Now add to both the unknown and standard 1 c.c. of saturated potassium persulphate and 4 c.c. of 3 N potassium sulphocyanate solution. Insert a clean rubber stopper, mix, and compare in a Duboseq colorimeter.

"Calculation.—As the 20 c.c. of filtrate taken represent 0.2 c.c. of the original blood, and the quantity of standard solution used contains 0.1 mg. of Fe, if the reading is made with the standard set at 20 mm., then 20 divided by the reading (*r*) of the unknown and multiplied by 50 will give the number of mg. of Fe in 10 c.c. of the blood examined. To obtain the percentage of hemoglobin, divide this number by 3.35, since hemoglobin contains 0.335 per cent of iron.

$$\frac{20}{R} \times 50 = \text{mg. of iron per hundred cubic centimeters of blood.}$$

$$\frac{20 \times 50}{R \times 3.35} = \text{percentage of hemoglobin in blood.}"$$

In some of the recent articles on blood iron the results are expressed as iron in milligrams per hundred cubic centimeters rather than as hemoglobin. Murphy, Howard, and Lynch³ have described an "iron index" in which the relation of the iron in milligrams to the red cell count in millions is calculated. Since, as I have indicated above, for every practical purpose, all the iron present is in the form of hemoglobin, I can see no reason for recording the results in terms of iron rather than of hemoglobin. Likewise, an "iron index" is the same as a color index since the relation of the iron to the cell count is again only another way of expressing the relation of the hemoglobin to the cell count.

Reich and Tiedemann¹² have suggested an "iron-volume index" which expresses the relation of the iron to the mass of packed red corpuscles. Since here again, the iron is really a measure of the hemoglobin, this is really a saturation index and serves only to confuse the issue by multiplying the various indices.

CONCLUSIONS

The iron content method is a simple and accurate method for determining the amount of hemoglobin in the blood, and the results check closely with those of the oxygen capacity method.

The iron method for the determination of hemoglobin deserves wider use in clinical laboratories for the checking of hemoglobinometers and other purposes.

For every practical purpose all the iron present in the blood is in the form of hemoglobin, so the results should be calculated as hemoglobin rather than as "blood iron."

The "iron index" is only another way of expressing the *color* index, and the "iron volume index" another way of expressing the *saturation* index.

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OBSERVATIONS UPON THE LIPOKRIT METHOD FOR THE DETERMINATION OF THE LIPOID CONTENT OF BLOOD*

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DURING the course of our work upon the experimental production of pulmonary fat embolism,¹ it was found that the tolerance for fat injected intravenously appeared to vary with the animal's nutrition and diet. Therefore it became necessary to control these experiments by determining the lipid content of the blood before and after the injection of fat. Accepted methods for the determination of blood lipoids, such as the Bang-Bloor chromate titration method or the gasometric method of Backlin, seemed unnecessarily detailed and laborious since we were primarily interested in relative variations of the total lipid content rather than exact quantitation of the individual lipid constituents. Our search for a simple procedure disclosed the Hemolipokrit method recently devised by Rückert² of Marburg, Germany. With a few modifications, this simple, volumetric micromethod has proved to be ideally suited to our needs, and we feel that it is of such potential value in investigative work upon "fats" that a detailed report of our experience with the method should be placed on record.

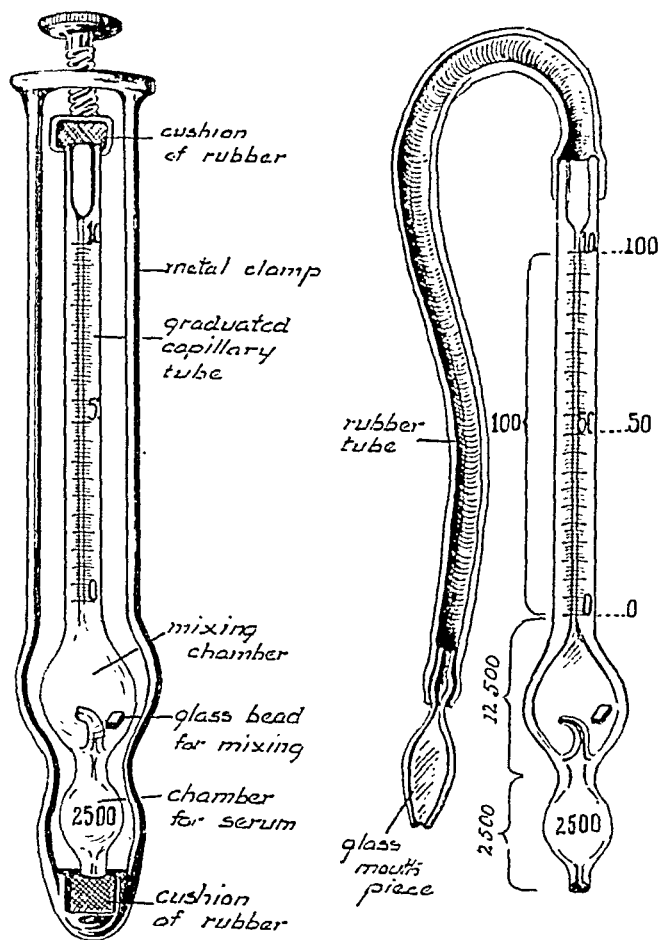
Throughout this report the term *lipoid* is used in accordance with the terminology employed by Peters and Van Slyke³ to denote the fats and fatlike substances in general, while the conjugated lipoids, such as the phosphatids and cerebrosides, are designated by the term *lipides*.

The principle of the Lipokrit method is essentially that of the Babcock method for determining the fat content of milk and cream. The proteins of the blood are changed from the colloidal state to one of molecular dissociation by means of sulphuric acid, permitting complete liberation of the lipoids by hydrolysis and centrifugation. Rückert found the optimum concentration of sulphuric acid for freeing lipoids from blood serum to be 70 per cent by weight when used in the proportion of five parts by volume of acid to one part of serum. He also devised the hemolipokrit pipette which made it possible to measure accurately the minute volume of lipoids derived from small amounts of blood (Fig. 1). The addition of a small amount of amyl alcohol to the sulphuric acid accelerates the separation of the fat droplets and facilitates their coalescence into a measurable column.

In testing the accuracy of this method, Rückert employed aqueous emulsions of the various lipoids and recovered them from the sulphuric acid, amyl alcohol mixture with an average error of only 0.004 gm. per cent. He found that

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neutral fats, fatty acids, cholesterol, and cholesterin esters separated immediately upon centrifugation, while phosphatids, such as lecithin, required an eleven-hour period of hydrolysis for complete liberation of their fatty acids. Mixed aqueous emulsions of equal parts of triolein and lecithin were found to yield an increasing volume of fat and fatty acids with hourly centrifugations until a maximum constant level was reached at the end of eleven hours (Fig. 2). The



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Fig. 1.—Sketch of the lipokrit pipette and clamp (after Rückert).

difference between the initial and final readings, therefore, represented the fatty acids derived from lecithin.

By applying the foregoing observations to the examination of blood serum, Rückert was able to establish a simple lipid partition by which the neutral fats, free fatty acids, cholesterol and cholesterin esters were obtained immediately upon centrifugation in the first fraction, while the various lipides were represented by their fatty acids in the second fraction obtained after eleven to twenty-hour hours of hydrolysis. In order to compute the volume of lipides

from their fatty acids in the second fraction, it was necessary to multiply the value for this fraction by the arbitrary factor 1.1964 obtained by assuming that lecithin, which bears a volumetric relationship of $\frac{739.4}{618}$ or 1.1964 to its fatty acid constituents, is the only important lipide of blood serum. The *total lipoid content* of a specimen of serum was then determined by adding the observed volumes-percentage of lipoids obtained in the first fraction to the computed volumes-percentage of lipides in the second fraction. Further differentiation of the lipoid partition may ultimately be possible. In serum of known high cholesterol content we have been able to demonstrate, with the polarizing microscope, a definite anisotropic layer at the base of the fat column in the lipokrit stem, but so far we have not succeeded in making consistent measurements of this layer.

At present there is no extensive work on the comparison of the results of the lipokrit method with those of other methods. Rückert,² and Leppien⁴ have made parallel volumetric and gravimetric determinations of the total lipoid content of serum. In these studies the results obtained by the volumetric method were converted from milliliters-percentage (ml.%) to grams-percentage (gm.%) by (A) applying an arbitrary specific gravity factor (obtained by assuming, as do Bloor⁵ and Backlin,⁶ that the mixture consists of two parts of fat to one of cholesterol) to the value for the mixed lipoids of the first fraction, and (B) by dividing the value of the lipides of the second fraction by the specific gravity of lecithin. The gravimetric values were obtained by extracting the lipoids with alcohol, ether, and petroleum ether and evaporating the filtrate to constant weight. The eight pairs of parallel determinations made by Rückert showed an average difference between volumetric and gravimetric results of 0.011 gm. per cent, with discrepancies ranging from 0.001 gm. per cent to 0.036 gm. per cent. In all but two, the volumetric value was the higher. In a similar series reported by Leppien the differences ranged from 0.003 gm. per cent to 0.066 gm. per cent, with an average discrepancy of 0.029 gm. per cent. All but three of these pairs showed a higher gravimetric value.

METHOD

*I. Apparatus.**—In devising the hemolipokrit pipette Rückert combined the essential features of the hematocrit tube, the erythrocytometer pipette and the Babcock bottle, adding a small inlet bulb one-fifth the volume of the mixing chamber, for measuring the proper proportion of serum. The serum bulb, the mixing chamber, and the graduated portion of the capillary stem bear a volumetric relationship to one another of 25:125:1. Accurate measurement of the sample of serum is facilitated by the addition of an "automatic" capillary tip which projects into the mixing chamber from the serum bulb (Fig. 1). The capillary stem is so calibrated that each of its 100 divisions represents 0.04 per cent of the capacity of the inlet or serum bulb. The actual volume of this bulb will vary slightly in different pipettes but the average capacity is 0.15 ml.

The hemolipokrit clamp is a great improvement over the ordinary hematocrit clip. The pipette can be held tightly enough in this clamp to permit violent shaking and high-speed

*The lipokrit pipettes are manufactured by Arno Hask, Jena, Germany. They are obtainable in the United States from the Cincinnati Scientific Company, 210 East Second Street, Cincinnati, O.

centrifugation without leakage. The difference in weight between the various pipettes and clamps is negligible thus eliminating the necessity for balancing before centrifugation.

II. Reagents.—(a) *Sulphuric Acid*: This reagent should be an aqueous solution of sulphuric acid, 70 per cent by weight with a specific gravity $\frac{20^{\circ} \text{ C.}}{4^{\circ} \text{ C.}}$ of 1.6105. Rückert found that more concentrated solutions oxidize the lipoids to a certain extent, while more dilute solutions are incapable of completely hydrolyzing the coagulated protein. We have confirmed these observations and, in addition, have found that a solution of sulphuric acid of greater specific gravity will give fictitiously high fat readings (detected by control tests with physiologic salt solution) by causing amyl alcohol to separate from the mixture. Since sulphuric acid (c. p.) varies somewhat in concentration, we have found it necessary to determine its specific gravity by means of a Westphal balance prior to making the calculations. It is also advisable to check the specific gravity of the solution which has been prepared to detect any possible error.

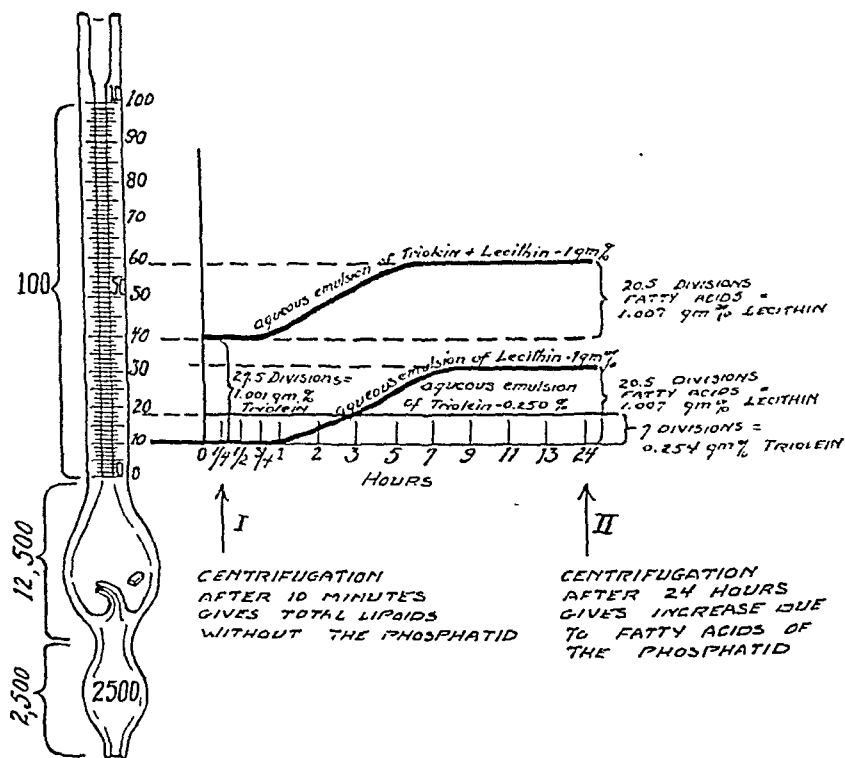


Fig. 2.—Sketch showing the time hydrolysis curve for aqueous emulsions of various lipoids (after Rückert).

(b) *Amyl Alcohol*: Chemically pure iso-amylie alcohol with a boiling point of 128° to 130° C. , and a specific gravity $\left(\frac{15^{\circ} \text{ C.}}{4^{\circ} \text{ C.}}\right)$ of 0.850 has been found more satisfactory for this method than the fusel oil ordinarily employed in milk analysis.

(c) *Contrast Stains*: In order to facilitate measurement of the lipoid column in the capillary stem of the lipokrit, Rückert stained the sulphuric acid with methylene blue and made the amyl alcohol a vehicle for a spirit soluble anilin yellow dye which is taken up by the fatty acid radicle. The clear, colorless fluid column in which the fatty layer is distinguishable only by the light reflex of the interface, is differentiated by this means into two sharply contrasting components—an opaque dark blue green column rising out of the mixing chamber and a supernatant pale yellow lipoid layer. The optimum concentration of these dyes in their respective solvents was found to be as follows: methylene blue (med-icinal) 0.08 gm. per 100 ml. of the 70 per cent sulphuric acid solution, and anilin yellow

0.15 gm. per 100 ml. of amyl alcohol. We have found that a saturated solution of secharlach R in amyl alcohol is a much more effective stain for the lipid layer than any anilin yellow dye which we have been able to obtain in this country. The bright red color imparted to the lipoids affords a much more striking contrast with the blue green color in the stem of the lipokrit pipette.

III. Technic.—(a) *Preparation of the Blood Sample:* The blood is taken from an artery or vein with small glass syringes which have been cleaned in sulphuric acid—potassium dichromate solution and dried with alcohol and ether, using rustless steel needles rendered fat-free by acetone and sterilized in the autoclave. In order to prevent hemolysis the needles and syringes must be absolutely dry. Two milliliters of blood is ample for duplicate de-

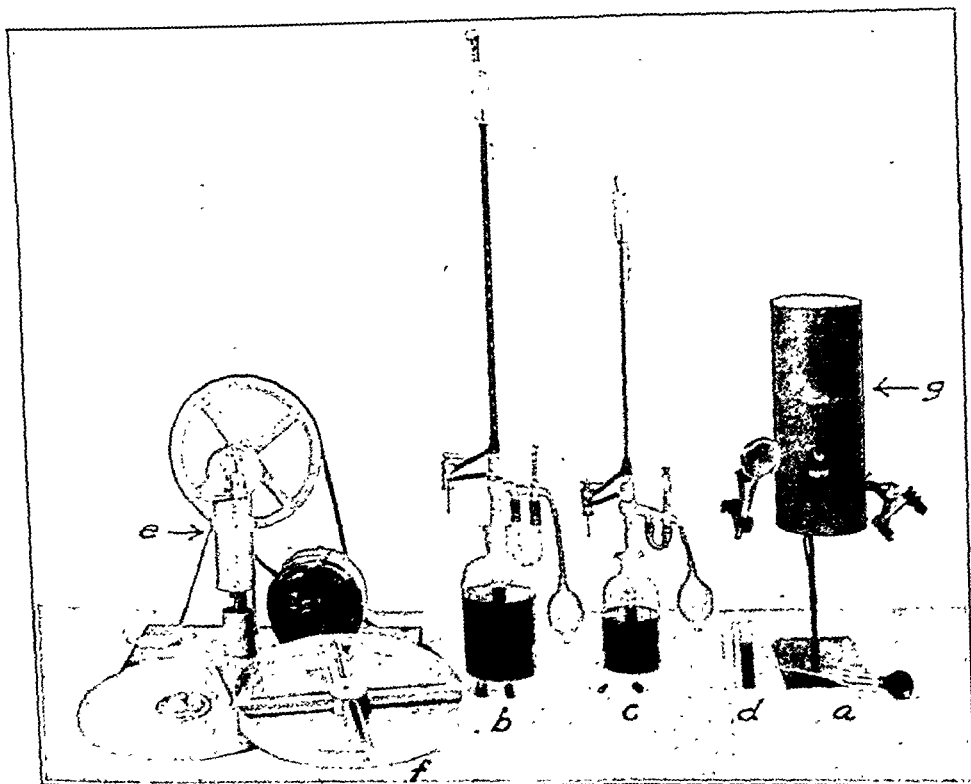


Fig. 3.—Photograph of the accessory apparatus. *a*, Capillary pipette for separating the serum. *b*, Automatic burette bottle for sulphuric acid, methylene blue solution. *c*, Automatic microburette bottle for amyl alcohol secharlach R solution. *d*, Glass stoppered weighing bottles for mixing the special reagent. *e*, Motor-driven shaking machine for the lipokrit pipettes. *f*, Special duralumin centrifuge head. *g*, Heat insulated slit lamp with magnifying glass and microburner attached.

terminations. The specimens are transferred to chemically clean, fat-free culture tubes 5 inches in length, and then centrifuged as soon as the clot has formed. We have found that centrifugation at approximately 1900 r.p.m. for five minutes in a No. 1C International centrifuge with head No. 233 fitted with the usual small cups gives the maximum yield of serum with a minimum demulsification of the fats. The supernatant serum is then transferred to another tube by means of a special large capillary pipette drawn from a 6-inch hard glass culture tube and fitted with an "asepto" rubber bulb (Fig. 3, *a*).

(b) *Preparation of the Reagent Mixture:* The sulphuric acid-amyl alcohol mixture is prepared by adding 1 volume of the amyl alcohol-secharlach R solution to 12.5 volumes of the sulphuric acid-methylene blue solution. Rückert found that when smaller proportions of alcohol are used the fat droplets tend to remain discrete and fail to coalesce properly upon

centrifugation, while larger proportions of amyl alcohol interfere with the hydrolysis of the proteins. Furthermore, the mixture should be *freshly* prepared for each set of determinations because amylene is formed on standing and will rise with the lipoids upon centrifugation and give fictitiously high fat readings. We have confirmed these findings to the extent of observing somewhat higher readings with the use of sulphuric acid-amyl alcohol mixtures which were twenty-four to seventy-two hours old. We have found it most convenient to keep these solutions in "all automatic" burette bottles (Fig. 3, *b* and *c*), using a microburette for the amyl alcohol, and protecting the sulphuric acid solution from atmospheric moisture by "trapping" the vents with calcium chloride tubes and stoppering them when not in use. With this equipment small amounts of reagents may be measured out and freshly mixed for each set of determinations with a minimum of waste and exposure, and a maximum of speed and accuracy. Approximately 0.75 ml. of the mixture is needed for each determination, consequently a series of 12 determinations would require 10 ml. of the sulphuric acid-methylene blue solution and 0.8 ml. of the amyl alcohol-scharlach R solution. These fluids should be thoroughly mixed by shaking vigorously for five minutes and warming to about 50° C. For

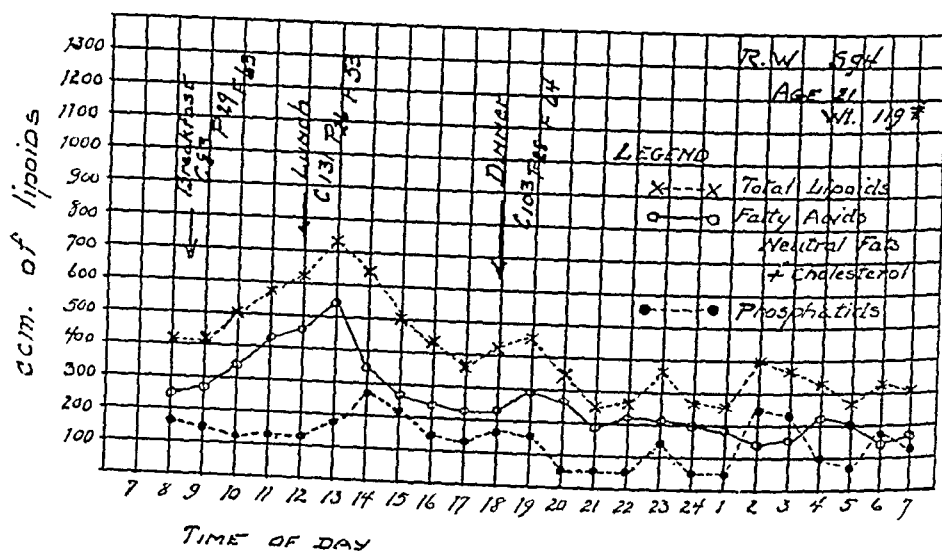


Fig. 4.—Alimentary lipemia curve of a normal adult.

this purpose narrow 4-inch glass-stoppered weighing bottles (Fig. 3, *d*) have proved most convenient. After mixing, the reagent should be allowed to stand for about twenty minutes or until it has cooled to room temperature and is free from bubbles. We have found that the premature use of this mixture causes a collection of foam in the lipokrit stem which interferes with the coalescence of the fat droplets. The reagent should, therefore, be prepared about thirty minutes before it is to be used.

(c) *Filling the Pipettes:* The serum should be agitated by gentle rotation of the tube to insure homogeneous distribution of the lipoids which sometimes come to the top of the serum during centrifugation. Violent shaking should be avoided because of the inconvenience of the resulting bubbles. The lipokrit pipette is then introduced into the tube and the serum is slowly drawn up into the measuring bulb until the automatic capillary tip is filled. The pipette is withdrawn, held horizontally while the end is wiped dry and then dipped into the reagent mixture. This is sucked up *quickly* through the inlet bulb to prevent coagulation of the serum in the automatic tip, and then drawn *very slowly* into the mixing chamber. During this process the pipette should be held vertically and twirled rapidly to and fro between the thumb and forefinger as the fluid rises. At least one minute should be consumed in filling this chamber, for if the process is hurried, dissociation of the coagulated protein will be sufficiently delayed to retard the liberation of the lipoids and lead to low readings

after the initial centrifugation. The reagent should be allowed to rise but halfway up the capillary stem, and when it has been adjusted at the 50 mark, the pipette should be withdrawn to the horizontal position, wiped dry, and fitted tightly into the lipokrit clamp. The apparatus is then shaken vigorously in a motor-driven device (Fig. 3, e) for five minutes. If the time intervals for filling and shaking have been properly observed, the fluid column will be found to have contracted with cooling to the level of the 10 or 15 mark at the base of the capillary stem, and this incompletely hydrolyzed portion may be allowed to run back into the mixing chamber without fear of losing any lipoid material at the inlet. Filling of the pipette is then completed by again drawing up reagent until the column reaches the 100 mark at the top of the stem. The objections to filling the pipette in one stage are, first, incomplete hydrolysis of the material in the stem, and second, contraction of the fluid column into the mixing chamber upon cooling. If the pipettes are filled properly in two stages, the fatty layer will always be found within the limits of the graduated portion of the capillary stem.

(d) *Centrifugation*: In his original publication of this method, Rückert specified centrifugation at a speed of 2,000 r.p.m. for ten minutes, but later in a personal communication to us he stated that he had found it necessary to increase the speed of centrifugation to 4,000

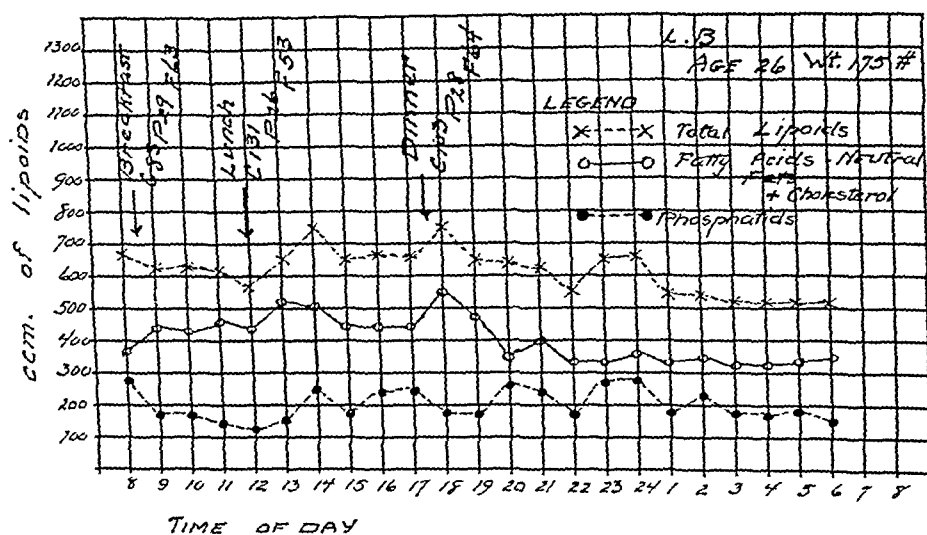


Fig. 5.—Alimentary lipemia curve of an obese adult.

r.p.m. in order to obtain the most constant and accurate results. We have found that the speed of centrifugation (the radius remaining constant) must be governed by the time hydrolysis curve of the lipides in relation to the total (elapsed) time necessary for filling, shaking, and centrifuging a given number of pipettes. By repeated determinations at various speeds on the same specimen of serum, with centrifugation for periods of five minutes at five-minute intervals, we have learned that the lipoids of the first fraction are usually liberated completely in from ten to thirty minutes after filling the pipettes, depending upon the degree of centrifugal force applied. We have also found that the hydrolysis of the lipides commences after thirty minutes have elapsed regardless of the speed and duration of centrifugation. It seems to be essential, therefore, that readings for the first fraction be completed within thirty minutes after filling the pipettes. If twenty minutes were required to fill and shake 4 pipettes then they should be centrifuged with sufficient force to liberate the entire first fraction in the remaining ten minutes, while if only 2 determinations were to be made, requiring but ten minutes for filling and shaking, there would be twenty minutes left for centrifugation and the separation could be accomplished at a slower speed with a centrifugal force which would be inadequate for a shorter interval. The "sedimentation-time" for lipid emulsions may be said to be approximately inversely proportional to the relative centrifugal force

(R. C. F.). Example: Using an International centrifuge No. 1C with head No. 233 and 2 small carriers (rotating radius of the center of gravity of the fluid mass being 15 cm.) at the maximum obtainable speed of 1,900 r.p.m., we were able to apply a centrifugal force of only 589,235 dynes/gm. (R. C. F. 601 gm./gm.), and complete liberation of the first lipid fraction required a twenty-minute period of centrifugation; while with the same centrifuge but a special duralumin head (radius 11.25 cm.) designed to reduce "windage," a speed of 3,500 r.p.m. was obtained, and the resulting centrifugal force of 1,502,156 dynes/gm. (R. C. F. 1,533 gm./gm.) was sufficient to separate these lipoids in about eight minutes. Theoretically this work could be accomplished in approximately one minute by an R. C. F. of 12,000 gm./gm. From these observations the following proportion may be stated, by means of which it is possible to estimate the *number of minutes of centrifugation* (T) required for any given combination of *speed* (N in r.p.m.) and *radius* (R in cm.), i.e.,

$$T : 1 :: 12,000 : 0.0000111N^2R$$

$$T = \frac{1,081}{\left(\frac{N}{1,000}\right)^2 R}$$

If T *plus* the time required for filling and shaking the desired number of pipettes is found to be thirty minutes or more, a greater centrifugal force will be needed. Although most of the determinations which we present in Tables I and II were made with an R. C. F. of 600 gm./gm. for fifteen minutes and a total (elapsed) time of twenty minutes for separating the first fraction, we are now convinced that in order to obtain comparable and uniform results *the initial centrifugation should be made consistently with an R. C. F. of 1,200 gm./gm., for a period of ten minutes and that the total elapsed time (from filling to reading) should be uniformly limited to twenty-five minutes.* The specified centrifugal force may be obtained with the following equipment:

MAKE OF CENTRIFUGE	MODEL	TYPE OF HEAD	SPEED
International	1C	Special (Fig. 3, f)	3,000 r.p.m.
International	1SB	Nos. 210; 220; 225	2,900 r.p.m.
International	2	Nos. 251; 240; 241	2,700 r.p.m.

(e) *The First Fraction: Readings.* After centrifugation the fluid column will usually be found to have contracted to the 70 or 80 mark on the lipokrit stem, and the coalesced, liberated lipoids, stained a bright scarlet, will be layered over the dark blue green reagent mixture at about this level. The length of the lipid column should be read promptly with the aid of a magnifying glass and a bright light. Because of the thermometer-like behavior of the sulphuric acid, a cool source of light is desirable, and we have found that readings are facilitated by the use of a heat-insulated slit-lamp (Fig. 3, g). Frequently a portion of the lipoids solidifies on cooling, and in order to obtain a clear-cut interface it is necessary to warm the portion of the lipokrit stem occupied by the fatty layer in the flame of a micro-burner. There is no appreciable expansion of the column of fat on heating. In cold weather it is advisable to do this routinely as quickly as possible, because slight warming of the bulbs from contact with the hands or lamp will make the fluid rise and leave solidified lipid particles behind thereby necessitating recentrifugation. Each division of the lipokrit scale is equivalent to 0.04 per cent of the volume of the serum taken into the pipette. It is possible to read accurately to one-tenth of one division. For example, a fatty layer occupying 9.6 divisions would represent a content of 0.384 ml. of neutral fat, free fatty acids, cholesterol and cholesterol esters per 100 ml. of serum. In view of the differences in specific gravity and variations in the relative proportions of these lipoids, we prefer to report the value for the first fraction in terms of the observed relative volume rather than attempt to transpose it into gravimetric units.

(f) *The Second Fraction: Time hydrolysis curve of the lipides.* After taking the initial readings, Rückert allowed the pipettes to stand for twenty-four hours in an incubator at 37° C., in order to obtain complete hydrolysis of the lipides. We have learned, however, from

parallel determinations at 37° C., and at room temperature (22° C. to 27° C.), that the rate and completeness of hydrolysis are not influenced appreciably by temperature within these limits. We have also observed that somewhat lower readings are frequently obtained after incubation at 37° C. due to the loss of small amounts of lipoid material which adhere to the glass above the capillary bore when the fatty layer is carried to the top of the stem on

TABLE I
VOLUME OF LIPOIDS IN RELATION TO THE PERIOD OF HYDROLYSIS

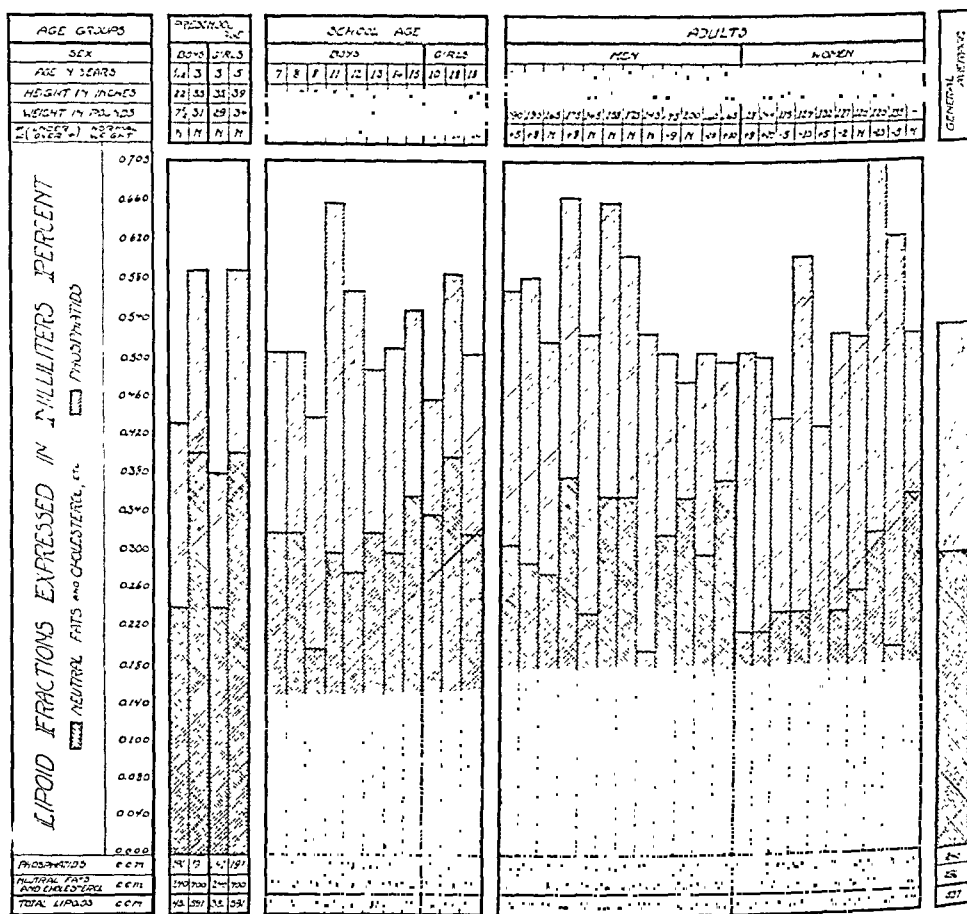
DONORS OF SERUM PERIOD OF FASTING, FOURTEEN HOURS		NO. OF LIPO- KRIT	VOLUME OF THE LIPOIDS (RECORDED IN DIVISIONS OF LIPOKRIT SCALE)				
			30 MIN- UTES	4 HOURS	8 HOURS	12 HOURS	24 HOURS
1	White male, aged 30, Ht. 65", Wt. 143 pounds. Interne. Hospital diet	211	5	10	11	11	11.5
		212	5	10	11	11	11.5
		213	5	10	11	11	11.5
2	White male, aged 26, Ht. 65", Wt. 145 pounds. Interne. Hospital diet	237	6	10	12	12	12
		239	6	10	11	12	12
		240	6	10	11	12	12
3	White male, aged 28, Ht. 70", Wt. 158 pounds. Interne. Hospital diet	231	9	14	14	15	15
		232	9	14	14	15	15
		233	9	13.5	14	14	15
4	White male, aged 29, Ht. 72", Wt. 175 pounds. Interne. Hospital diet	231	9	13	14	14	14
		232	9	14	15	15	15
		233	9	13	14	14	14
5	White male, aged 25, Ht. 72", Wt. 175 pounds. Interne. Hospital diet	234	7	10.5	12	12	12
		235	7	11	12	12	12
		236	7	10.5	12	12	12
6	White female, aged 47, Ht. 64", Wt. 250 pounds. Hy- pertension. 1,200 cal. diet	235	7	13	14	14	14.5
		236	7	13	14	14	14
		237	7	12	13	14	14
7	White female, aged 48, Ht. 65", Wt. 225 pounds. Obst. jaundice. Hospital diet	234	10	12	15	15.5	15.8
		235	10	13	15	15.5	16
		236	10	13	14.5	15.3	16
8	White female, aged 24, Ht. 65", Wt. 140 pounds. Preg- nancy, term. Hospital diet	231	20	26	26	27	27
		232	21	26	26	27.5	27.5
		233	20	26	26	27	27
9	White female, aged 24, Ht. 55", Wt. 166 pounds. Tox- emia, preg. Nephritic diet	211	10	10	11	12.8	12.9
		212	9	10	12	13	13
		213	9	10	13	13	13
10	White male, aged 52, Ht. 65", Wt. 127 pounds. Catar. jaundice. Fat-free diet	222	2.7	13	--	17	17.5
		223	2.4	12.2	--	16	17
		224	2.5	12	--	17.5	17.5

TABLE II

DIVISIONS →		UNITS									
	↓	0	1	2	3	4	↑ 5	6	7	8	9
T E N S	0	0	0.048	0.096	0.144	0.191	0.239	0.287	0.335	0.383	0.431
	1	0.478	0.526	0.574	0.622	0.670	0.718	0.765	0.831	0.861	0.909
	2	0.957	1.005	1.053	1.100	1.148	1.196	1.244	1.292	1.340	1.387
	3	1.435	1.483	1.531	1.579	1.627	1.674	1.722	1.770	1.818	1.866
	4	1.914	1.961	2.009	2.057	2.105	2.153	2.201	2.248	2.296	2.344
	5	2.392	2.440	2.488	2.536	2.583	2.631	2.679	2.727	2.775	2.823

warming. Consequently, we prefer to carry out this process at room temperature. Furthermore, we believe that there is not sufficient difference between twelve-hour and twenty-four-hour readings to justify depriving the laboratory of the use of the lipokrit pipettes for more than twelve hours. Evidence for this opinion is presented in Table I of 10 determinations in triplicate (Table I). After the twelve-hour period of hydrolysis, the fatty acids derived from lipides are added to the lipoids of the first fraction by a second period of centrifugation which should be identical with the first.

IV. Calculations.—The value for the reading obtained after the twelve-hour period of hydrolysis does not represent the total lipid content of the



the first fraction. These calculations are greatly facilitated by the use of Table II prepared by Rückert, in which the ml. per cent of lipides (phosphatids), computed from their fatty acid components, is given for any number of divisions of the lipokrit scale from 1 to 59.

V. *Results*.—Fig. 6 shows the volume of lipoids in the blood serum, after fourteen hours of fasting, as determined by the lipokrit method in a series of 37 normal patients.

Since only a small quantity of blood is needed for each set of determinations made by this method, the rate and degree of absorption of fat from the alimentary canal can be studied easily and without injury to the patient. Several normal adults volunteered for such studies. All of these patients were given the same diet of known composition. The lipoids of the blood were determined at hourly intervals for twenty-four hours. The results of two of these series of determinations are shown in the accompanying curves (Figs. 4 and 5). A more detailed report of similar studies will be presented at some later time.

SUMMARY

The lipokrit method is a simple volumetric micromethod for the determination of the lipoid content of blood serum.

Slight alterations of the original method of Rückert with certain refinements of technic have made it possible for us to obtain consistently uniform results.

The lipoid content of the blood serum, after fasting for about fourteen hours, appears to be fairly constant regardless of the age or sex of the individual.

Two curves showing the typical increase in the lipoids of the blood which occurs several hours after each meal are presented.

NOTE: The authors wish to express their appreciation to Dr. Glenn E. Cullen, Director of Laboratories of the Children's Hospital Research Foundation, for his kind cooperation and many valuable suggestions during the course of this work.

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THE MODIFIED GRAM STAIN OF MUCH*

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B. MÖLLERS, in the *Handbuch der pathogenen Mikroorganismen* (1930), states that "in the various forms of human tuberculosis one finds only the acid-fast form of the tubercle bacillus, or one attributes only to this form a diagnostic value." In a previous paper¹ by me it was seen that the classical tubercle bacillus has no genuine Gram property as long as the organism is acid-fast; moreover, that it cannot have this property because of the presence of the acid-fast lipoids. It was found further that when the tubercle bacillus was completely deprived of its acid-fast material, it became gram-negative. H. Much,² however, claimed to have found, with the aid of a modified Gram staining technic, a pathogenic granular nonacid-fast form of the tuberculosis virus, a form hitherto unknown. According to his findings this organism, when stained by his method, is found to consist either of a very short, beaded fragmented rod or of isolated granules, or heaps of granules, all of which are nonacid-fast and gram-positive.

When one studies the Gram technic of Much, it will be seen that the latter employs two carbol dyes such as are used in acid-fast staining, viz., carbol-fuchsin 3 parts, carbol methyl violet 1 part. Moreover, Much's technic requires prolonged cold staining up to forty-eight hours. In order to give the method a semblance of the Gram stain, the organisms are then exposed to hot iodine solution for five minutes. This is followed successively with 5 per cent nitric acid for one minute, 3 per cent hydrochloric acid for ten seconds, and finally with equal parts of 95 per cent alcohol and acetone. These latter are acid alcohol acetone decolorizers that are even more powerful in their action than those applied in the classical Ziehl-Neelsen acid-fast method, in which only 3 per cent hydrochloric acid in 70 per cent alcohol is employed. Consequently, any microorganisms retaining the Much dyes after being treated with these decolorizers must be considered not merely alcohol-fast as required of gram-positive organisms but acid-fast as well. Hence, this led me to conclude that the so-called modified Gram stain of Much is really an acid-fast staining technic, and that the Much granules are in reality acid-fast bodies. In order to observe the action of Much's staining method on acid-fast tubercle bacilli, some experimental work was carried out in which this staining technic was analyzed into its separate procedures.

Experiment 1.—*Purpose:* To determine the action of the Much's modified Gram staining technic on untreated acid-fast tubercle bacilli. Exposure time, forty-eight hours. The tubercle bacilli stained an intense purplish blue especially the granules which stand out like cocci either in rows or in clusters. One could scarcely note any difference between these and Much's granules as found in the pus from cold abscesses. None of the bacilli stained red, the purplish blue color predominated (Fig. 1).

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Experiment 2.—Purpose: To determine the effect upon color retention of untreated tubercle bacilli stained with aniline gentian-violet as employed in the standard Gram stain, of the decolorizing agents employed in the Much staining technic.

A. Tubercle bacilli were stained on slides at room temperature for one minute with aniline gentian-violet. Gram's iodine was applied for five minutes hot as in Much's technic. Decolorization with 5 per cent nitric acid for one minute, 3 per cent hydrochloric acid for ten seconds, acetone alcohol until color ceases to come off.

Results: Organisms were well stained. They retained their color after use of Much's decolorizers.

B. Tubercle bacilli were treated as under A, with one-minute exposure to cold Gram's iodine solution.

Result: Organisms were equally well stained as in A.

C. Tubercle bacilli were treated as under A, but without iodine.

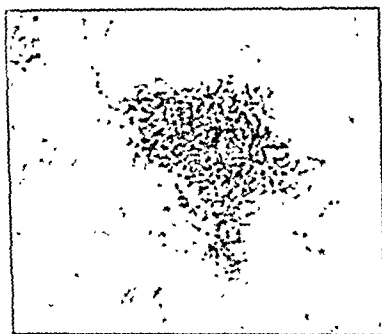


Fig. 1.—Tubercle bacilli of the beaded type which have been stained according to Much's technic, resembling Much's "granules." Note intergranular bridges, one of the requisites of true "Much granules."

Result: The organisms retained stain in spite of lack of application of iodine when the Much acid acetone alcohol decolorizing solutions were employed.

Experiment 3.—In this experiment the Much modified Gram stain was analyzed by staining untreated intact pulverized living tubercle bacilli with the separate components of this technic in order to ascertain whether all the steps of the Much technic tend to increase its efficiency in tinctorially demonstrating the tubercle bacillus. The results of the experiment are recorded in Table I.

Comment on Experiment 3: It will be seen in B of Table I that when the entire system of decolorizers employed in Much's staining technic is employed, the intensity and the quality of red of the carbolfuchsin are greatly altered so that the tubercle bacilli are scarcely recognizable. On the other hand as seen in A of the table the methyl violet is but little altered in these respects by the same decolorizers.

From the above staining analysis, by the process of elimination it appears that the principle offender in causing alteration of the color quality and intensity of the carbolfuchsin is the nitric acid. This acid changed the brilliant red of the carbolfuchsin either to a pale lavender or entirely decolorized the tubercle bacillus of this stain whether iodine was used or not. The action of nitric acid on methyl violet, however, is not so marked. Hence, the carbolfuchsin adds practically nothing to the end-staining result since the nitric acid nearly decolorizes this stain when applied to the tubercle bacillus in the course of the Much technic.

Moreover, it will be seen that the tubercle bacillus stains as well and better without, as with, iodine. When iodine was applied after the carbol methyl violet, the former produced an annoying granular precipitate which obscured the tubercle bacilli and simulated the granules of the latter. It causes a change in color quality and staining intensity of the carbolfuchsin.

It is consequently evident that in employing the Much staining technic

TABLE I

TUBERCLE BACILLI STAINED WITH:				
A. CARBOL METHYL VIOLET			B. CARBOLFUCHSIN	
DECOLORIZER	WITH IODINE	WITHOUT IODINE	WITH IODINE	WITHOUT IODINE
1. HNO ₃ , HCl, Acetone alcohol	Intensely stained	Intensely stained	Practically not stained	Poorly stained. Nearly decolorized
2. HNO ₃ , Acetone alcohol	Well stained	More intensely stained than with iodine	Practically not stained	Practically not stained
3. HNO ₃ , HCl	Stain precipitated in small granules, obscuring tubercle bacilli	No precipitate. Bacilli well stained	Very poorly stained, a lavender color	Very poorly stained, a lavender color
4. HNO ₃	So much granular precipitate as to obscure bacilli	No precipitate. Bacilli well stained	None bright red; some pink	Not bright red. Lavender. Most organisms not stained or poorly so
5. HCl, Acetone alcohol	No precipitate. Not as intensely stained as (1) above	No precipitate. Bacilli well stained	Practically not stained	Well stained. Bright red
6. HCl	Much granular precipitate obscuring bacilli	No precipitate. Bacilli well stained	Not distinctly stained	Stained bright red
7. Acetone alcohol	No precipitate. Bacilli well stained	Slight granular precipitate. Bacilli not obscured by precipitate. Latter well stained	None bright red. Poorly stained	Well stained, red
8. Acetone	Granular precipitate. Bacilli well stained	No precipitate. Bacilli well stained	Lavender tubercle bacilli. None red. Faintly stained	Stained red
9. Alcohol	Much granular precipitate. Bacilli well stained	No precipitate. Bacilli well stained	No stained tubercle bacilli at all	Well stained, red

we are not dealing even with a modified Gram stain but with an acid-fast stain for the following reasons:

- The component dyes of the Much stain are typical acid-fast stains.
- The Gram's iodine adds nothing to the value of the Much staining technic since typical tubercle bacilli will stain as well without iodine as with it, in fact, somewhat better without it (see Table I).
- Tubercle bacilli stained with Much's stain will retain the latter (whether treated with iodine or not) not only after decolorization with alco-

hol, as is to be expected of a gram-positive organism, but also after decolorization with nitric and hydrochloric acids, and acetone alcohol. Hence the stain is an alcohol acid-fast stain and not a Gram stain.

d. In these experiments the tubercle bacillus has been used to determine the acid-fast property of the Much stain, since this organism is generally considered the most acid-fast one of that group. If, then, it be correct, as asserted by Much, that the so-called Much "granules" are nonacid-fast gram-positive bodies representing an atypical form of the tuberculosis virus, he should have employed a method of staining other than that devised by him, since his is an acid-fast stain and the bodies stained therewith are, consequently, acid-fast "granules" (they are not always granules, but frequently show connecting intergranular bridges or bacillary structure), resembling, in reality, the granular forms of the typical tubercle bacillus.³

Experiment 4.—The purpose of this experiment was to ascertain whether the method of concentrating tubercle bacilli affects unfavorably the staining of these organisms by the Much technic. In this method of concentrating tubercle bacilli (and "Much granules") from sputum and pus from cold abscesses, strong alkalis (0.6 per cent sodium carbonate and antiformin which contains sodium hydroxide) are applied for forty-eight hours in order to liquefy the tenacious sputum. The organisms so liberated are then concentrated by centrifugation and applied in the alkaline sediment to slides, dried and stained.

With this in view pulverized intact acid-fast tubercle bacilli were treated with the above concentrating solutions, centrifuged after forty-eight hours and half of them applied to slides without being washed free from alkalis; the other half was repeatedly washed in distilled water until neutral to litmus and then applied to slides and dried. The unwashed bacilli were then stained as follows:

1. With Much's carbofuchsin and carbol methyl violet for forty-eight hours, hot Gram's iodine five minutes, decolorized with nitric acid, hydrochloric acid, acetone alcohol.

Results: The tubercle bacilli were stained indistinctly a slate blue color.

2. With carbofuchsin forty-eight hours, hot Gram's iodine five minutes, decolorized with nitric acid, hydrochloric acid, acetone alcohol.

Result: Tubercle bacilli were stained, but not distinctly, a pale lavender instead of a bright red.

3. With cold carbol methyl violet forty-eight hours, hot Gram's iodine five minutes, decolorized with nitric and hydrochloric acids and acetone alcohol.

Result: Tubercle bacilli stained a dark gray-blue, with the granules well stained, the intergranular substance often was not stained; if stained at all, the latter took the dye faintly as a rule. These tubercle bacilli appear very much like the typical Much "granules."

4. With carbofuchsin, hot, five minutes; decolorized with acid-alcohol, counterstained with methylene blue.

Result: The tubercle bacilli were stained more distinctly than under (2) but not a brilliant red.

Comment: From the first part of Experiment 4 it will be seen that the alkalis employed to concentrate the tubercle bacilli in sputum or pus from cold abscesses, previous to the application of the Much "granule" staining technic, are distinctly detrimental to the staining of tubercle bacilli with Much's carbofuchsin and carbol gentian violet both as to intensity and color quality. The tubercle bacilli treated with these alkalis do not stain typically bright red with carbofuchsin. Only carbol methyl violet, after application of these alkalis, colors the tubercle bacillus with moderate intensity.

The washed bacilli were stained as follows:

1. With hot carbolfuchsin five minutes (as in 4 above), hot iodine solution five minutes and Ziehl-Neelsen acid alcohol decolorizer.

Result: The tubercle bacilli were stained reddish, but not a bright red.

2. With the same as in (1) without the iodine.

Result: These tubercle bacilli stained a brilliant red.

Comment: The second part of Experiment 4 demonstrates the following:

a. That if the alkalis used in concentrating tubercle bacilli are removed by washing from the material to be stained, they will not interfere with the staining property of the carbolfuchsin of the Ziehl-Neelsen stain, but if not properly removed they may render the organisms unrecognizable or difficult to find due to poor staining. This may account for some of the failures to detect tubercle bacilli when the concentration method is employed. This method would not, however, interfere seriously with the discovery of the organisms if stained with the intensely blue staining carbol methyl violet of the Much method. This may consequently account for the greater efficiency of the Much staining method in detecting rare tubercle bacilli after application of the above alkaline concentration method.

b. The addition of iodine in the Much technic alters to a considerable extent the bright red color of the carbolfuchsin but not greatly the color quality of the carbol methyl violet.

Experiment 6.—A freshly isolated culture of virulent streptococci was stained according to Much's technic in order to ascertain the effect of this stain upon a typical Gram positive nonacid-fast organism.

Result: Streptococci were completely decolorized by the acid acetone alcohol decolorizers of the Much technic. Only occasional faint outlines of organisms were visible.

Comment: The Much stain is not a Gram stain but acts as an acid-fast stain when applied to known gram-positive organisms.

DISCUSSION

Much² maintained that he had discovered a new form of nonacid-fast tuberculosis virus which he claims to have demonstrated by a staining technic devised by him and designated a modified Gram stain. This virus so stained appeared either as purple isolated granules or as heaps of granules, or as beaded, bandlike structures. Unless these granules were connected by a faint bluish or reddish band, it was said, they should not be considered to be true Much granules.³ This claim of Much to the discovery of an atypical tuberculosis virus has been supported by some and called into question by many, so that B. Möllers⁴ in the *Handbuch der pathogenen Mikroorganismen* states recently that the question is still unsettled. Much, however, in his autobiography recently states that his discovery of the virus now is an established fact of science. Although this assertion may well be questioned, the true nature of the "granules" has not been gone into in this paper. In the work here presented, merely the staining method of Much has been called into question.

First, it is claimed by Much that his staining method is a modified Gram staining technic. The work recorded in this article has shown that this claim of Much is incorrect. That first of all his staining technic is not a modified

Gram stain; and second, that it is an acid-fast and not a nonacid-fast stain. In order to prove the former of these contentions gram-positive organisms were employed. These were shown by Much's method to lose their original stains (carbol genitan violet and carbolfuchsin) upon application of his decolorizers following application of hot Gram's iodine solution. According to Much's method then, these gram-positive organisms would have to be considered gram-negative. This would prove incompatible with our conceptions of a Gram stain. The evident reason for the decolorization of these gram-positive organisms is the presence in the Much decolorizers of two acids, besides alcohol and acetone. For a microorganism to be considered gram-positive it must resist alcohol decolorization after application of iodine solution. If a microorganism resists decolorization with acid, even though iodine solution has been applied, it is an acid-fast organism. Such is the case with the Much technic. It is an acid-fast technic and merely the inclusion by it of Gram's iodine solution which adds nothing to its efficacy does not make it a Gram stain. In order further to prove this assertion acid-fast tubercle bacilli were stained with the Much technic and they retained the Much stain after the application of HCl and HNO₃ decolorizers.

Moreover, it was seen in a previous article¹ that the tubercle bacillus in its acid-fast pathogenic state has no true Gram property, because it requires no iodine to enable it to retain its original stain and is not only alcohol but also acid-fast. That, consequently, its acid-fast property precluded the possibility of its possessing genuine gram-positivity. Hence the retention on part of the tubercle bacillus of the Much stain indicated not gram-positivity but acid-fastness.

Thus it will be seen from the experiments in this paper that the Much stain is not a Gram stain, but is actually a powerful acid-fast stain (carbol-fuchsin and carbol methyl violet) that is applied over a prolonged period of time (forty-eight hours), the decolorizers are two strong mineral acids (HCl and HNO₃), followed by acetone and alcohol. The iodine was found in the above experiments to be not only unnecessary but detrimental in the retention of the original stain. It was, moreover, found that when the Much staining technic was applied to a pure culture of living beaded acid-fast tubercle bacilli on slides, the latter presented the characteristics of Much "granules" (see Fig. 1). Consequently, it appears from this work that the bodies called Much granules are really acid-fast entities, and not nonacid-fast gram-positive bodies as claimed. No other known nonacid-fast organisms will remain gram-positive in the face of acid alcohol as was seen in Experiment 6.

Why, when stained with Much's carbolfuchsin carbol gentian-violet stain, do the Much bodies appear deep purplish blue and not reddish? This occurs, as seen in this work, for the following reasons:

1. The gentian-violet obscures the fuchsin due to its darker coloration.
2. The alkali of the method of concentrating tubercle bacilli in sputum and pus interferes with the bright red of the fuchsin, not only of the Much method, but also of the Ziehl-Neelsen method. This probably accounts for the fact that Much "granules" have more frequently been demonstrated than tubercle bacilli with the Ziehl-Neelsen stain in pus from cold abscesses.

3. The nitric acid changes the color quality of the fuchsin to a lavender shade but has no apparent effect on the methyl violet.

In order to demonstrate that the so-called Much "granules" are actually a tuberculosis virus apart from the standard tubercle bacillus, it will be necessary for Much or the other advocates of this staining method to employ other than an acid-fast stain to demonstrate nonacid-fast bodies. As to what the real nature of the Much "granules" may be was not a part of the above work to ascertain, but these bodies greatly resemble granular tubercle bacilli or fragments thereof (see Fig. 1).

CONCLUSIONS

1. The Much staining technic is not a true or a modified Gram stain because it employs acid decolorizers instead of only alcohol as for the classical Gram stain.

2. It is an acid-fast stain because in it carbol dyes and acid alcohol decolorizers are employed. When the most important pathogenic acid-fast micro-organism, the tubercle bacillus, is stained according to Much's technic, the latter is shown to be a strong acid-fast stain for this organism whose visibility is superior to that of tubercle bacilli stained with the Ziehl-Neelsen.

3. When the Much stain is applied to the granular form of the tubercle bacillus, the latter resembles, in fact is, indistinguishable morphologically from Much's granules. This work has not, however, endeavored to determine whether the Much "granules" are really tubercle bacilli or not.

4. If the "granular" bodies that are found in certain sputa and in pus from cold abscesses by the Much staining method are actually a different form of tuberculosis virus, one that is nonacid-fast and gram-positive, then this technic cannot be employed to demonstrate such a virus since it is an acid-fast staining procedure.

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AN EVALUATION OF THREE METHODS FOR THE DEMONSTRATION OF TUBERCLE BACILLI FOR USE IN HOSPITAL ROUTINE^{*}

A SPECIAL STUDY OF SEVERAL SIMPLE CULTURE MEDIUMS

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IN RECENT years the efficacy of the culture method for the isolation and identification of the tubercle bacillus from various types of tuberculous material has received considerable notice in the literature. Corper and Uyei^{1, 2} have reviewed the work of previous authors and published reports comparing the guinea pig inoculation method with the culture method. The question of the superiority of either method is still under discussion. Recent authors, Herrmann, Hansmann, and DeCapito,^{3, 4} report superior results with guinea pig injections. At the same time, Norton, Thomas, and Broom⁵ advocate the use of the culture method on the grounds of economy as well as efficiency.

The guinea pig inoculation method has been criticized because many guinea pigs contract tuberculosis spontaneously or have internal lesions which resemble those caused by the tubercle bacillus. Howell and Schultz⁶ report an epizootic among guinea pigs due to a paratyphoid B bacillus which caused lesions closely resembling tubercles in the spleen and liver. Magath and Feldman⁷ in a recent complete review of the subject conclude that with reasonable care spontaneous tuberculous infection of guinea pigs can be avoided. Cummings⁸ has reported the incidence of spontaneous tuberculous infection in laboratory animals in Saranac, a tuberculosis center.

In the present study an attempt has been made to compare direct smear, guinea pig inoculation and culture methods for the demonstration of the tubercle bacillus in the routine specimens received in the laboratory of a large hospital. This study differs from many previous ones in that no attempt was made to obtain material from clinically tuberculous patients. In hospital laboratories, the bacteriologist is more often concerned with ruling out a possible tuberculous infection rather than confirming a clinical diagnosis of tuberculosis. In many instances the diagnosis may be obscure or deferred until laboratory reports are complete. Therefore, the time element is important and must enter into a study of methods for the demonstration of the tubercle bacillus. As Feldman and Magath report,⁹ animal inoculation remains the most satisfactory method for determining the virulence of an isolated acid-fast organism. However, if the condition of the patient indicates to the clinician the pathogenicity of the "unknown" causative organism, a quick laboratory report of an acid-fast bacillus of typical tubercle bacillus morphology and growth is of more value than a belated

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report of a virulence test. In this respect the culture method supplements identification of the tubercle bacillus by direct smear.

In some instances even a prolonged search fails to demonstrate tubercle bacilli in certain specimens. In that case, other methods of demonstration must supplement direct smears. Pottenger^{10, 11} states that a search for five minutes yields 80 per cent of positive diagnoses obtainable in a fifteen-minute search; and that with careful work a longer search than fifteen minutes is not justified by the additional results. Von Huth and Lieberthal¹² state that smears were negative and cultures positive for the tubercle bacillus on 50 of 200 urines from tuberculous kidneys. They claim absolute reliability for the culture method. Corper,¹³ realizing the difficulty of the quick demonstration of tubercle bacilli by direct smear when only small numbers of the bacilli are present in a specimen, has recently devised a tissue substrate culture method to supplement identification of the organism by direct smear.

TABLE I

THE DEMONSTRATION OF TUBERCLE BACILLI BY DIRECT SMEAR, GUINEA PIG INOCULATION AND CULTURE METHODS

CLINICAL DIAGNOSIS	MATERIAL	NUMBER OF SPECIMENS	DIRECT SMEAR	GUINEA PIG INOC.	CULTURE
Positive	Urine	1	+	+	+
	Tissue	3	+	+	+
	Pus	1	+	+	+
	Urine	3	-	-	+
	Pus	2	-	-	+
	Urine	2	+	-	-
	Spinal fluid	1	-	-	-
	Pus	1	-	+	+
	No. Positive		7	6	11
Negative	Urine	8	-	-	-
	Pus	3	-	-	-
	Fluid exudate	1	-	-	-
	Stools	2	-	-	-
	No. Positive		0	0	0
Unknown	Stools	3	-	-	-
	Fluid exudate	16	-	-	-
	Tissue	1	-	-	-
	Spinal fluid	3	-	-	-
	Sputum	3	-	-	-
	Pus	8	-	-	-
	Fluid exudate	1	-	+	-
	Urine	1	-	-	+
	Fluid exudate	1	-	-	+
	Tissue	1	-	+	+
	Spinal fluid	2	-	+	+
	Urine	1	-	+	+
	No. Positive		0	5	6

In the present study 69 specimens (Table I) were examined by direct smear, guinea pig inoculation and culture methods. Table I outlines these cases according to the clinical diagnoses. Direct smears were examined for thirty minutes. Fourteen specimens were obtained from clinically tuberculous patients. Of these, 5 specimens were positive by all three methods. In 2 cases, smears were positive and the results were not confirmed by either cultures or guinea pig injections; when examined after two months both cultures and

guinea pigs showed no evidence of tubercle bacilli. In 5 cases, demonstration of the tubercle bacillus was accomplished when both smears and guinea pig inoculations gave negative results. In one case, all three methods gave negative results, and in another case both guinea pig inoculations and culture methods yielded a positive result when direct smears had been negative. Of these specimens from clinically known tuberculosis cases, 7 were proved positive by direct smear, 6 by guinea pig inoculation and 11 by culture methods.

Fourteen specimens were obtained from clinical cases not diagnosed as tuberculosis. In addition to the negative clinical diagnosis, routine cultures showed the presence of other organisms. The 14 specimens were negative for tubercle bacilli when examined by all three methods.

Forty-one specimens were sent to the laboratory with no clinical diagnoses on the records. In four instances guinea pig inoculations and cultures gave a positive result although the direct smears were negative. In two other cases, the guinea pig injections and direct smears were negative, but tubercle bacilli were isolated by cultures. In one instance the guinea pig inoculation alone showed a positive result. In this series a total of 5 specimens gave positive results by guinea pig inoculation, 6 by culture and none by direct smear. Thirty-four specimens were negative for tubercle bacilli when tested by all three methods.

Table II summarizes these results. From these figures the inadequacy of direct smears for the demonstration of tubercle bacilli and the superiority of culture methods to the guinea pig inoculation method is obvious.

Many types of culture mediums for the cultivation of the tubercle bacillus have been devised. Most of them have a base of eggs or potatoes and are reinforced with glycerol. Corper and Uyei^{14, 15} have reported excellent results with crystal violet potato cylinder medium. The efficiency of the egg mediums of Miraglia,¹⁶ Hohn,¹⁷ Lubenau,^{18, 19} Dorset,²⁰ Sweany and Evanoff,²¹ and more recently Herrold,²² Feldman,²³ Woolley and Petrik,²⁴ has also been favorably

TABLE II

A COMPARISON OF DIRECT SMEAR, GUINEA PIG INOCULATION AND CULTURE METHODS FOR THE POSITIVE DEMONSTRATION OF THE TUBERCLE BACILLUS IN CLINICALLY POSITIVE, NEGATIVE AND QUESTIONABLE MATERIAL

METHOD OF DEMONSTRATION	NUMBER OF SPECIMENS	NUMBER OF POSITIVE SPECIMENS	PERCENTAGE OF POSITIVE RESULTS
Culture	69	17	24.6
Direct smear	69	7	10.1
Guinea pig inoculation	69	11	15.9

reported in the literature. Koch's²⁵ original serum medium has given way to synthetic mediums such as Long's.^{26, 27} A simple medium with asparagin as a source of nitrogen has been devised by Lowenstein.^{28, 29, 30} The asparagin is substituted for meat extracts which, he says, will not inhibit the growth of the tubercle bacillus. It is doubtful whether the ideal medium for the cultivation of the tubercle bacillus has yet been devised.

In routine laboratory procedure it is obviously impossible to employ a large number of mediums. In order to determine the most satisfactory ones, cultures were made on several well-known mediums using Corper's, Hohn's and Miraglia's

as controls. One specimen with a positive direct smear showed growth of tubercle bacilli in seven days on both Hohn's and Miraglia's mediums, but seventy-five days were required for growth to appear on Lubenau's egg medium. Four specimens were cultivated on Herrold's egg yolk agar. No growth was obtained on this medium or on the controls. Known cultures of tubercle bacilli grew very poorly when transferred onto Herrold's medium. In spite of careful precautions, tubes of Herrold's medium dried out early in the long period of incubation necessary for the growth of tubercle bacilli. This medium also showed a high percentage of contaminated cultures. These experiences substantiate the statement of Magath and Feldman⁵ that Herrold's reports are not complete. Woolsey,³¹ however, has found Herrold's medium an excellent means of demonstrating tubercle bacilli.

Woolley and Petrik's egg medium is rather complicated to prepare, but supports excellent growth and, therefore, may be used for stock cultures of tubercle bacilli. Of 36 specimens cultured on this medium and on the controls, two were positive on the controls and one on Woolley and Petrik's medium after twenty-five days. Nineteen of these same specimens were cultivated on 5 per cent glycerol agar, long a standard medium. The same specimens which showed growth of tubercle bacilli in twenty-five days on Woolley and Petrik's medium became positive in fifty days on the glycerol agar. Glycerol agar may be used for stock cultures of tubercle bacilli, but it dries out rapidly and often becomes contaminated.

Thirty-one specimens were cultured on Lowenstein's medium. Three of these specimens were positive on the controls, none on Lowenstein's medium. Eight blood samples were included on this series. Five of them were from patients with acute arthritis or rheumatic fever, one of them from a case of typhoid, and two from tuberculous patients. All eight specimens gave negative results on the controls as well as on Lowenstein's medium. This is contrary to Reitter and Lowenstein's³² report that a high percentage of positive growth of tubercle bacilli is obtained when blood specimens from arthritic, rheumatic and tuberculous patients are cultured by Lowenstein's method on his medium. Although Abt³³ claims reliability for Lowenstein's method, Cummings³⁴ reports negative blood cultures on both Hohn's and Lowenstein's mediums. Weatherall³⁵ was unable to substantiate Lowenstein's work. Wallgren³⁶ states the necessity for specialized technic if positive blood cultures are to be obtained. Additional study of Lowenstein's medium in the present investigation proved that it supported only scanty growth of tubercle bacilli even when inoculated with large amounts of stock cultures.

For standard work, therefore, these various mediums were rejected in favor of Corper's, Hohn's and Miraglia's mediums. These do not require elaborate preparation and prove to be efficient.

MEDIUMS

Hohn's medium was made according to standard methods.¹⁷

Corper's medium was made according to his standard method.¹⁴

The preparation of Miraglia's medium is described here because this method differs in its extreme simplicity from standard methods described elsewhere.

Miraglia's medium originally consisted of seven egg yolks in 100 c.c. of a 5 per cent glycerol beef broth. More recently, in accordance with the work of Feldman,²³ a 6 per cent glycerol water solution has been substituted for the broth. However, no change in results has been noticed.

The eggs are sterilized by immersing in 60 per cent alcohol for ten minutes. Sterilize the hands, break the eggs quickly, separate the whites from the yolks, and drop the yolks into a large sterile flask with a wide mouth. If the flask is sufficiently large, the egg yolks will break as they hit the bottom and thus eliminate stirring, an obvious element in contamination. Previously sterilized and measured broth is then added, the mixture well shaken, tubed under sterile conditions and coagulated in an Arnold or oven at 90° C. Medium made by this simple method has always been found to be sterile.

PROCEDURE

All specimens except stools, sputums, and tissues were cultured and if sterile were inoculated without further treatment onto tubercle bacillus mediums. Contaminated specimens must be treated with some reagent. The more common ones in use are 5 per cent oxalic acid, 6 per cent sulphuric acid, 15 per cent sulphuric acid, 3 per cent sodium hydroxide, 3 per cent hydrochloric acid, 3 per cent acetic acid, or antiformin. In the present study one-half of the specimens were treated with 6 per cent sulphuric acid and the other half with 5 per cent

TABLE III

A COMPARISON OF CORPER'S, HOHN'S AND MIRAGLIA'S MEDIUMS FOR THE ISOLATION OF THE TUBERCLE BACILLUS

DIAGNOSIS	MATERIAL	NUMBER OF SPECIMENS	CORPER'S MEDIUM	HOHN'S MEDIUM	MIRAGLIA'S MEDIUM
Positive	Tissue	1	+	+	+
	Urine	4	+	+	+
	Urine	1	+	-	-
	Pus	1	+	-	-
	Pus	1	+	+	-
	Urine	1	-	+	+
	Pus	1	-	+	+
	Tissue	1	-	+	+
	Urine	1	-	-	-
	Sputum	1	-	-	-
	Blood	2	-	-	-
	Spinal fluid	1	-	-	+
	Tissue	1	-	-	+
	Pus	1	-	-	+
	Tissue	1	-	+	-
	No. Positive		8	10	11
Negative	Urine	7	-	-	-
	Sputum	1	-	-	-
	Blood	2	-	-	-
	Spinal fluid	1	-	-	-
	Pus	1	-	-	-
	Stools	3	-	-	-
	No. Positive		0	0	0
Unknown	Stools	2	-	-	-
	Pus	9	-	-	-
	Spinal fluid	2	-	-	-
	Fluid exudate	15	-	-	-
	Sputum	2	-	-	-
	Urine	32	-	-	-
	Pus	1	-	+	-
	Fluid exudate	1	+	-	+
	Urine	1	+	+	+
	Urine	1	-	+	-
	Urine	1	-	-	+
	No. Positive		2	3	3

oxalic acid. Both reagents appeared to give equally efficient results. Fifteen per cent sulphuric acid was used to kill the contaminating organisms in stools.

Stools, sputums, tissues, and contaminated materials were treated with equal parts of the reagent for one-half hour at 37° C. Solid specimens were ground in a mortar with the reagent. The treated material was then centrifugalized for one-half hour, washed with sterile physiologic saline solution, recentrifugalized and the sediment inoculated onto the mediums with a pipette. Blood specimens were treated according to Lowenstein's method before inoculation. Four or five drops of sediment were smeared over the surface of each tube of medium and at least four tubes of each medium were inoculated for each specimen. The tubes were sealed by pipetting approximately 2 c.c. of melted paraffin on each plug; the tubes were capped with tinfoil. All cultures were incubated in covered tin cans for four months at 37° C. in an incubator before being discarded. Smears were made periodically and again just before the cultures were discarded.

Table III summarizes the results obtained using Corper's, Hohn's, and Miraglia's mediums. Nineteen specimens were received from patients with clinical diagnosis of tuberculosis. Fifteen specimens yielded positive growth on at least one medium. Five were positive on all three mediums; four were negative on all three. Two of these four negative specimens did not produce tuberculosis when injected into the guinea pigs. Two were blood specimens which were not inoculated into guinea pigs. Three specimens were positive on Miraglia's alone, two on Corper's alone, and one on Hohn's alone. One specimen gave a positive growth on both Hohn's and Corper's mediums and three were positive on both Hohn's and Miraglia's. In this series a total of eight specimens gave positive growth on Corper's medium, ten on Hohn's medium, and eleven on Miraglia's medium.

Fifteen specimens from patients with a clinical diagnosis other than tuberculosis gave no growth of tubercle bacilli on the three mediums.

Sixty-seven specimens were received with clinical diagnoses unknown. Of these, 62 were negative. One specimen gave positive results on all three mediums, one each on each of the three mediums, one each on each of the three mediums alone, and one, on Hohn's and Miraglia's only.

These results would lead to the conclusion that Corper's medium is less favorable to the growth of the tubercle bacillus than are the other two mediums. Corper's medium, however, showed contaminations in only 5.2 per cent of tubes inoculated, Hohn's in 13.6 per cent, and Miraglia's in 19.4 per cent.

For further comparison of mediums a second series of 68 specimens were cultivated on Corper's medium and on Miraglia's medium. Table IV summarizes the results obtained in this study.

Five specimens were from tuberculous patients. Tubercle bacilli from one of these grew on both Corper's and Miraglia's mediums. One was positive on Miraglia's alone; 3 were negative on both mediums. Of the total 5, one was positive on Corper's and 2 on Miraglia's medium.

Six specimens tested were obtained from patients with a clinical diagnosis other than tuberculosis. All 6 specimens were negative for tubercle bacilli when cultured on both mediums.

Fifty-seven specimens were received with diagnoses deferred or unknown. Fifty-four were negative when cultivated on both mediums. One was positive on Miraglia's alone, and 2 were positive on both Miraglia's and Corper's

TABLE IV

A COMPARISON OF CORPER'S AND MIRAGLIA'S MEDIUMS FOR THE ISOLATION OF THE TUBERCLE BACILLUS

DIAGNOSIS	MATERIAL	NUMBER OF SPECIMENS	CORPER'S MEDIUM	MIRAGLIA'S MEDIUM
Positive	Tissue	1	+	+
	Tissue	1	-	+
	Tissue	1	-	-
	Spinal fluid	2	-	-
	No. Positive		1	2
Negative	Tissue	1	-	-
	Blood	3	-	-
	Pus	1	-	-
	Fluid exudate	1	-	-
	No. Positive		0	0
Unknown	Fluid exudate	8	-	-
	Blood	2	-	-
	Tissue	1	-	-
	Stools	6	-	-
	Spinal fluid	1	-	-
	Sputum	4	-	-
	Urine	30	-	-
	Pus	2	-	-
	Urine	1	+	+
	Spinal fluid	1	+	+
	Spinal fluid	1	-	+
	No. Positive		2	3

mediums. A total of 2 were positive on Corper's and 3 on Miraglia's medium. In this series the percentage of contaminated cultures was approximately 5 per cent for Corper's and 10 per cent for Miraglia's medium.

From these results it is apparent that the egg mediums are more sensitive than Corper's medium for the isolation of tubercle bacilli. However, since Corper's medium becomes contaminated much less often than Hohn's or Miraglia's, it should be included as a control medium in routine cultures.

As has been emphasized, the time element is important in the laboratory demonstration of tubercle bacilli. Positive results were obtained by guinea pig injection occasionally in twenty-one days, but the average period was sixty days. Cultures on egg mediums were quite often macroscopically positive in seven days; the average number of days in which positive cultures were ob-

TABLE V

METHOD	MINIMUM NUMBER OF DAYS FOR GROWTH TO APPEAR	AVERAGE NUMBER OF DAYS IN WHICH GROWTH APPEARED
Positive guinea pig inoculations	21	60
Positive cultures on Corper's medium	25	38.5
Positive cultures on Hohn's medium	7	27.7
Positive cultures on Miraglia's medium	7	30.7

tained on Hohn's medium was 27.7 days and on Miraglia's 30.7 days. This substantiates the previous statement that the low percentage of contaminated cultures obtained with Corper's medium is the most important reason for its use in routine work. These statements are summarized in Table V.

SUMMARY AND CONCLUSIONS

A comparison of direct smear, guinea pig inoculation and culture methods for the demonstration of tubercle bacilli in various types of specimens indicated culture methods to give a greater percentage of positive results more quickly than either of the other two methods.

A study of various mediums for the isolation of the tubercle bacillus proved the three simple mediums, Corper's, Hohn's and Miraglia's, to be suitable for routine diagnostic cultures. Hohn's and Miraglia's mediums gave larger numbers of positive results in less time than did Corper's medium. Either of these two mediums may be used with equal efficiency. Corper's medium, however, is sufficiently sensitive and becomes contaminated less often than the egg mediums and, therefore, should be included in routine culture methods.

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MODIFIED TECHNIC FOR MAKING WRIGHT'S BLOOD STAIN*

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THE object of this note is to suggest a slight modification in the technic for making the usual Wright's stain of a blood film. The apparatus requires two staining jars with covers. The reagents are pure methyl alcohol and Wright's stain† diluted up to 30 per cent with distilled water.

One staining jar is filled with pure methyl alcohol. The second contains the 30 per cent solution of Wright's stain. The blood film is made and dried in the usual manner. It is then immersed in methyl alcohol for at least five minutes. The slide is then placed in the stain for from two or three minutes up, depending upon the intensity of the stain desired. The slide is then washed as usual.

Using this technic there is no precipitation on the slide. It has been found that this method is economical. The stain can be used repeatedly instead of only once as in the usual technic. When the fluid level of the reagents becomes low more is added until there is enough to cover the slide. It is not necessary to discard any of the stain. Even though the solutions are used for several months staining takes place with good results. Even though the slide is in the methyl alcohol for an hour or more, there is no change in the staining properties of the cells.

*From the Pyengyang Union Christian Hospital.

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†The Wright's stain used in this study was prepared by dissolving one tabloid (Burr's Welcome Co.) in 10 c.c. pure methyl alcohol.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

DIPHTHERIA: A Differential Culture Medium for *B. diphtheriae* and *B. pseudodiphtheriae* (Un Nuovo Metodo Culturale per la Differenzazione Del Bacillo Difterico Dai Bacilli Pseudodifterico), Bruschetti, G. Diagn. E. Techn. di Lab. 4: 212, 1933.

On this medium the diphtheria bacillus, fermenting glucose, produces a greenish color; in the absence of glucose-fermenters the medium shows a yellowish tinge. Because of its lack of specificity, the method is not reliable for direct isolation.

Preparation of Medium.—

1. Immerse a fresh egg in 1:1000 bichloride of mercury solution for 1 hour.
2. Place the egg "in alcohol" (per cent not stated) for 2 hours.
3. Dip one end of the egg in ether which is then burned off in the flame.
4. With a sterile pipette puncture the shell and transfer the contents to a sterile, glass-stoppered bottle (150 c.c.).
5. Add an equal volume of sterile "creamless" (skimmed?) milk and shake vigorously for ten minutes.
6. To 60 c.c. of this mixture add the following with sterile precautions.

Beef serum, sterile	60 c.c.
Glycerin	5 c.c.
2% sterile solution of glucose	5 c.c.
2% sterile solution malachite green	5 c.c.

7. Mix by shaking five minutes, place in sterile tubes, slant, and coagulate in an inspissator.

VACCINES: Comparison of Relative Values of the Intracutaneous Skin Test and of the Pathogen-Selective Culture in Selecting Bacteria for Vaccines From Mixed Cultures, Solis-Cohen, M. Am. J. Clin. Path. 3: 305, 1933.

There is practically no difference between the reactions produced by intracutaneous injections of organisms that are able to grow in the patient's fresh, whole, coagulable blood and the reactions produced by organisms that are killed by his blood.

There seems to be no correspondence between absence of bactericidal power in the blood of the host against a given organism and production in that host of a positive reaction by the intracutaneous injection of such organism.

There probably is no relationship between hypersensitiveness in the host to the exogenous and endogenous toxins of a given organism and the pathogenicity of such organism for that host.

It is questionable whether intracutaneous tests can identify, in a mixed culture, the bacteria that are infecting the patient.

Therefore, intracutaneous skin tests are probably unreliable for selecting bacteria in the preparation of vaccines.

MILK, A Study of the Brom-Thymol-Blue Reaction in Freshly Drawn, Bryan, C. S. Am. J. Pub. Health 23: 721, 1933.

Apparently there are normal physiologic factors that are responsible for a variation in the pH of freshly drawn milk.

The test cannot be used alone to detect cases of mastitis, since positive reactions were obtained in animals that were always free of mastitis. No definite cause is given for these

positive reactions. The work of Hucker, et al., was confirmed, indicating that a negative brom-thymol-blue reaction does not assure freedom from mastitis.

The brom-thymol-blue reaction is of value as an aid in determining the relative disease or freedom from mastitis of a herd. If a large number of positive reactions are obtained it is a good indication that something is wrong in the herd but the individual cases cannot be detected.

PHOTOGRAPHY: The Invisible Photographic Screen, Harding, F. R. J. Biol. Photo. Assn. 1: 203, 1933.

At the Children's Hospital has been developed what might be termed the "invisible screen," because the patient never sees it. It consists of a piece of thin celluloid the same size as the negative that is to be used. This sheet of celluloid is scored with cross-hatched lines one-quarter inch apart. The scoring is done with a scribing tool or the point of a knife. Care must be taken not to cut too deeply or the celluloid will split if it is bent. After the scoring is completed, red or black wax crayon is rubbed into the lines, and the sheet polished with a soft cloth.

To use the screen, lay it on the glass of the printing machine, place the negative on top of it and make the print in the usual manner. The result will be a positive covered with fine white, crossed lines.

As a guide in registering the screen with the negative, a thin, true vertical line is made on the background. This line should show somewhere on the negative. When ready to print, simply register the background line with one of the screen lines.

TUBERCLE BACILLI, Nutrient Quality of Eggs for Growing, Corper, H. J., and Cohn, M. L. Am. J. Hyg. 18: 1, 1933.

Quantitative evaluation of inspissated egg yolk as contrasted with the inspissated white of egg shows the latter to be a poor nutrient for small numbers of tubercle bacilli as compared to the former. The inspissated yolk has also proved slightly superior to the inspissated whole egg.

The addition of broth is not essential to the nutrient qualities of whole egg or egg yolk media. The inspissation of egg or egg yolk media is beneficial to the nutrient qualities and insures sterility as compared to the use of raw unsterilized whole egg or egg yolk.

The addition of a non-nutrient substance such as agar does not introduce any beneficial action upon the nutrient qualities of whole egg or egg yolk media, and if added in too great amount diminishes their nutrient value for small numbers of bacilli.

The addition of water up to 25 per cent to egg yolk before inspissation serves to fulfil the purpose of preventing drying of this medium during the long period of incubation required for the growth of tubercle bacilli.

Mixing of two good nutrients for tubercle bacilli such as egg yolk and potato does not serve to enhance the nutrient qualities of either of these substances but may change the type of colony.

Large plantings of tubercle bacilli grow on a wide range of acidity in good nutrient media such as egg yolk, but a few bacilli require an acidity of narrower range approximating the neutral point.

The value of dyes intimately mixed, with a nutrient such as egg yolk is mainly to assist in the ease of finding early colonies, but they must be used cautiously in a concentration which exerts no tuberculostatic action in graded planting tests. Several pigments and dyes are adapted to this purpose.

SERUM CALCIUM, In Normal Boys, Molitch, M., Weinstein, S., and Cousins, R. F. Am. J. M. Sc. 186: 378, 1933.

The serum calcium findings on 511 normal boys, between the ages of eight and twenty years, range from 7.6 to 12.5 mg. per cent. Of the total number reported, 33 were found

below and 1 above the extreme normal limits of 8.6 and 12 mg. per cent. Disregarding the highest and lowest 5 per cent of distribution, the normal limits are from approximately 8.5 to 10.8 mg. per cent. No progressive decrease was found with age in this group. Nationality and race showed no influence on serum calcium metabolism.

BLOOD CHEMISTRY: Serial N P N Studies and Their Prognostic Significance in Acute Coronary Occlusion, Steinberg, C. L. *Am. J. M. Sc.* 186: 372, 1933.

From a study of 16 cases it is concluded that (a) a rising nonprotein nitrogen or (b) a nonprotein nitrogen that remains elevated in the blood plasma is of ill omen.

More attention should be paid to blood chemistry studies during coronary occlusion as a prognostic aid.

A lowering of the blood pressure and diminution in urine output will not alone explain the rise in nonprotein nitrogen in acute coronary occlusion.

HYPERGLYCEMIA, Hypertension, Obesity and, Musser, J. H., and Wright, D. O. *J. A. M. A.* 101: 420, 1933.

A group of thirty obese hypertensive women exhibited a marked lowering of sugar tolerance. A second group of fat individuals without hypertension are shown not to have a hyperglycemia. It seems reasonable to assume that there is no one factor definitely responsible for the combination of obesity, hypertension and hyperglycemia unless it is obesity. The reduction in weight is often associated with a lowering of the blood pressure and a return of a sugar tolerance curve to normal.

PNEUMONIA, Value of Skin Test With Type-Specific Capsular Polysaccharide in the Serum Treatment of Type I Pneumococcus Pneumonia, Francis, T., Jr. *J. Exper. Med.* 57: 4, 617.

Skin tests were made with Type I S.S.S. in 53 cases of Type I pneumococcus lobar pneumonia, 48 of which were treated with antipneumococcus Type I serum. In all but 1 of the 46 recovered cases a positive, immediate skin reaction was obtained at about the time of recovery. In 7 fatal cases reactions were consistently negative, even in the presence of circulating type-specific antibodies.

The skin test has proved to be an extremely valuable guide to serum therapy, and a definite prognostic aid. The test has distinct advantages over the agglutination reaction in that it is not merely an index of circulating antibodies. When positive, it invariably denotes that recovery has begun; when negative, it indicates further serum therapy. The mechanism of the positive skin test is closely related to that operative in recovery from pneumonia, and is apparently the resultant of antibody and tissue activity.

TUBERCULOSIS, Gastric Examination in Pulmonary Tuberculosis With Negative Sputum Examination, Ulmar, D., and Ornstein, G. G. *J. A. M. A.* 101: 835, 1933.

A search was made of the gastric contents for tubercle bacilli in pulmonary tuberculosis. The mechanism of bronchial peristalsis is demonstrated whereby bronchial contents are raised to the level of the larynx without the mechanism of cough. The importance of this mechanism in raising sputum from the lungs is amply shown by the fact that, in a series of 287 cases in which there were repeated negative sputum examinations, approximately 20 per cent yielded tubercle bacilli on examination of the gastric contents. In some of those positive cases there was no previous coughing and expectoration. The importance of this method of investigation becomes increasingly significant when the normal cough mechanism has been interfered with as, for example, in pneumothorax or thoracoplasty cases. The simplicity of the examination, which consists merely in the usual concentration technic on the aspirated gastric contents, makes the examination so easy as to warrant its routine use in all suspected sputum negative cases.

HEPATIC FUNCTION: Clinical Values of Levulose and Galactose Tolerance Tests for, Radhakrishna, M. V. Ind. J. M. Res. 21: 141, 1933.

From this study it is concluded that the levulose tolerance test gave positive results in a greater number of cases with liver disease than in those without evidence of hepatic disorder. In some cases with definite evidence of liver disease the test gave negative results, and hence it is concluded that the test cannot be considered to be of diagnostic importance in any individual case.

The variable results obtained by the levulose tolerance test in cases of cirrhosis of the liver are considered to be due to the regeneration of the hepatic tissue. It is pointed out that a negative test in these cases cannot exclude liver disease, as it only indicates that a sufficient amount of actively functioning liver tissue is still left to maintain the carbohydrate metabolism. The test becomes positive only when there is diffuse destruction of the hepatic parenchyma together with failure of regeneration.

Though the galactose tolerance test of Bauer has its own advantages, it is misleading to depend on the urinary findings alone and the total amount of galactose excreted in the urine is too variable to consider it as a guide to hepatic efficiency.

ENDO MEDIUM, Nature of Reaction of Colon Organism on, Margolena, L. A., and Hansen, P. A. Stain Techn. 8: 131, 1933.

Considerable confusion in the understanding of Endo's reaction is caused by the fact that a faint coloration may be produced by weak acids and by oxidations, both causing a restoration of the fuchsin color.

The viewpoint of Neuberg and Nord that the cultivation on the Endo medium is a trapping procedure (Abfangverfahren) for acetaldehyde is confirmed.

The typical reaction of *Bacterium coli* is caused not by lactic acid, but by the intermediate product acetaldehyde which is fixed by sodium sulphite.

The color produced in the reaction is not restored fuchsin, which is shown by the fact that it cannot be shaken out with ether when picric acid has been added beforehand.

TYPHOID CARRIERS, Serological Diagnosis of, Wyllie, J. Am. J. Hyg. 78: 393, 1933.

In any agglutination method using living bacterial suspensions the pitfalls are due to the S R variation and to the H O variation. The former variation is easily detected by the appearance of spontaneous agglutination (granular in type) in increasing strengths of saline controls. A slight granularity in 0.85 per cent saline is more marked in 1.7 per cent and more so in 3.4 per cent and 6.8 per cent saline. When this occurs the stock culture is replated on agar and a smooth colony picked off. In this way the colony form is taken as an indication of smoothness or roughness and this is afterward examined for physiologic smoothness or roughness.

The latter variation (i.e., H O) is guarded against by subculturing the "H" variants on agar slants containing some water of condensation. O variants may be subcultured on dry agar slopes. The agar medium must be carefully prepared as outlined on pages 395 and 396 and the use of motility tubes containing semisolid agar ensures maximum development of the H-receptors. Felix (1931) recommends however that agar stabs for maintaining stock cultures over long periods should not contain muscle sugar, hence this agar should be prepared from meat extracts instead of fresh meat.

In this study of the serologic reactions of typhoid carriers by Felix' method, the sera were examined qualitatively with living bacillary suspensions of "H" and "O" strains of *B. typhosus* and *B. para-typhosus* A and B. In the large majority of these sera "O" agglutinins were found exclusively. The typhoid H suspension of the Oxford Standards Laboratory, however, revealed the presence of "H" agglutinins in nearly all of these sera.

The use of living typhoid and paratyphoid strains necessitated particular care to maintain the cultures in the optimum phase. The results show that sufficient differences in the type and degree of agglutination in the three classes of sera studied—from typhoid

carriers, from typhoid fever cases and from individuals receiving prophylactic inoculation—justify the opinion that an additional serological procedure is available to assist in distinguishing carriers from normal and from inoculated individuals.

TUBERCULOSIS, Effect of Exercise on the Leukocyte Count in Pulmonary, Kaminsky, J.
Am. J. M. Sc. 186: 484, 1933.

In 50 tuberculous patients leucocyte counts were made after one hour's rest in bed and the results compared with those of blood counts taken after some exercise.

Thirty-eight cases showed an increase in their total counts following exercise (increases of more than 2,000 cells were obtained in 19 cases); 12 cases showed a decrease in their total counts taken after exercise.

Striking variations were noted in some of the differential counts taken after exercise; increases in the cells of some types exceeding 100 per cent were encountered.

The value of performing leucocyte counts under basal conditions in the study of cases with pulmonary tuberculosis is emphasized.

RETICULOCTES: A New Method of Enumeration, Schleicher, E. M. Am. J. Clin. Path. 3: 395, 1933.

Solution A

Neutral potassium oxalate	1.0 gram
Sodium chloride c. p.	0.85 gram
Distilled water	100 c.c.

Solution B

Brilliant cresyl blue	1.0 gram
Sodium chloride c. p.	0.85 gram
Distilled water	100 c.c.
Chloretone (as a preservative)	1.0 gram

In a conical tipped centrifuge tube place 25 parts of Solution A and 5 parts of Solution B, mix (since a precipitate forms when the solutions are mixed, it is better to make up a larger volume, filter and place the proper amount of the mixed solution in the tube and proceed), and add several drops of blood. After thoroughly mixing, permit to stand ten to twenty minutes, then centrifugalize for twenty to thirty seconds at a moderate speed. The supernatant fluid is pipetted off until a layer of fluid approximately equal to the depth of the sediment remains. Mix the sediment well with the supernatant liquid, draw up in the pipette and discharge one drop near the end of a perfectly clean glass slide.

Spread the stained drop with the edge of a coverslip square 18 mm. and draw the film to about 6 cm. from the starting point.

The underlying principle of the method involves the Relative Reticulocyte Distribution (R.R.D.) throughout the smear. To know the relative distribution of reticulocytes is important, since it is technically not possible to make a blood film manually so that the distribution of erythrocytes and reticulocytes is equal throughout the preparation. It has been determined, however, that the distribution varies in a definite way when the above technic of preparing smears is followed each time.

The R. R. D. is found as follows: Divide a piece of paper into 10 columns and mark them 1 to 10. Adjust a 4x eyepiece to a tube-length of 190 mm., using an oil immersion objective 1.8 mm. x 95 N.A. 1.30 in order to obtain the specific microscopic working field. Any other combination giving the same field may be used.

The R. R. D. in the film will usually be found to show about four variations in concentrations as follows: (1) extremely high, (2) high, (3) low, (4) extremely low or absent.

Place the objective on the upper edge of the film near the starting point, that is, the extreme left border of the film. Moving the slide upward in a vertical line, count several

HEPATIC FUNCTION: Clinical Values of Levulose and Galactose Tolerance Tests for, Radhakrishna, M. V. Ind. J. M. Res. 21: 141, 1933.

From this study it is concluded that the levulose tolerance test gave positive results in a greater number of cases with liver disease than in those without evidence of hepatic disorder. In some cases with definite evidence of liver disease the test gave negative results, and hence it is concluded that the test cannot be considered to be of diagnostic importance in any individual case.

The variable results obtained by the levulose tolerance test in cases of cirrhosis of the liver are considered to be due to the regeneration of the hepatic tissue. It is pointed out that a negative test in these cases cannot exclude liver disease, as it only indicates that a sufficient amount of actively functioning liver tissue is still left to maintain the carbohydrate metabolism. The test becomes positive only when there is diffuse destruction of the hepatic parenchyma together with failure of regeneration.

Though the galactose tolerance test of Bauer has its own advantages, it is misleading to depend on the urinary findings alone and the total amount of galactose excreted in the urine is too variable to consider it as a guide to hepatic efficiency.

ENDO MEDIUM, Nature of Reaction of Colon Organism on, Margolena, L. A., and Hansen, P. A. Stain Techn. 8: 131, 1933.

Considerable confusion in the understanding of Endo's reaction is caused by the fact that a faint coloration may be produced by weak acids and by oxidations, both causing a restoration of the fuchsin color.

The viewpoint of Neuberg and Nord that the cultivation on the Endo medium is a trapping procedure (Abfangverfahren) for acetaldehyde is confirmed.

The typical reaction of *Bacterium coli* is caused not by lactic acid, but by the intermediate product acetaldehyde which is fixed by sodium sulphite.

The color produced in the reaction is not restored fuchsin, which is shown by the fact that it cannot be shaken out with ether when picric acid has been added beforehand.

TYPHOID CARRIERS, Serological Diagnosis of, Wyllie, J. Am. J. Hyg. 78: 393, 1933.

In any agglutination method using living bacterial suspensions the pitfalls are due to the S R variation and to the H O variation. The former variation is easily detected by the appearance of spontaneous agglutination (granular in type) in increasing strengths of saline controls. A slight granularity in 0.85 per cent saline is more marked in 1.7 per cent and more so in 3.4 per cent and 6.8 per cent saline. When this occurs the stock culture is replated on agar and a smooth colony picked off. In this way the colony form is taken as an indication of smoothness or roughness and this is afterward examined for physiologic smoothness or roughness.

The latter variation (i.e., H O) is guarded against by subculturing the "H" variants on agar slants containing some water of condensation. O variants may be subcultured on dry agar slopes. The agar medium must be carefully prepared as outlined on pages 395 and 396 and the use of motility tubes containing semisolid agar ensures maximum development of the H-receptors. Felix (1931) recommends however that agar stabs for maintaining stock cultures over long periods should not contain muscle sugar, hence this agar should be prepared from meat extracts instead of fresh meat.

In this study of the serologic reactions of typhoid carriers by Felix' method, the sera were examined qualitatively with living bacillary suspensions of "H" and "O" strains of *B. typhosus* and *B. para-typhosus* A and B. In the large majority of these sera "O" agglutinins were found exclusively. The typhoid H suspension of the Oxford Standards Laboratory, however, revealed the presence of "H" agglutinins in nearly all of these sera.

The use of living typhoid and paratyphoid strains necessitated particular care to maintain the cultures in the optimum phase. The results show that sufficient differences in the type and degree of agglutination in the three classes of sera studied—from typhoid

carriers, from typhoid fever cases and from individuals receiving prophylactic inoculation—justify the opinion that an additional serological procedure is available to assist in distinguishing carriers from normal and from inoculated individuals.

TUBERCULOSIS, Effect of Exercise on the Leukocyte Count in Pulmonary, Kaminsky, J.
Am. J. M. Sc. 186: 484, 1933.

In 50 tuberculous patients leucocyte counts were made after one hour's rest in bed and the results compared with those of blood counts taken after some exercise.

Thirty-eight cases showed an increase in their total counts following exercise (increases of more than 2,000 cells were obtained in 19 cases); 12 cases showed a decrease in their total counts taken after exercise.

Striking variations were noted in some of the differential counts taken after exercise; increases in the cells of some types exceeding 100 per cent were encountered.

The value of performing leucocyte counts under basal conditions in the study of cases with pulmonary tuberculosis is emphasized.

RETICULOCYTES: A New Method of Enumeration, Schleicher, E. M. Am. J. Clin. Path. 3: 395, 1933.

Solution A

Neutral potassium oxalate	1.0 gram
Sodium chloride c. p.	0.85 gram
Distilled water	100 c.c.

Solution B

Brilliant cresyl blue	1.0 gram
Sodium chloride c. p.	0.85 gram
Distilled water	100 c.c.
Chloretone (as a preservative)	1.0 gram

In a conical tipped centrifuge tube place 25 parts of Solution A and 5 parts of Solution B, mix (since a precipitate forms when the solutions are mixed, it is better to make up a larger volume, filter and place the proper amount of the mixed solution in the tube and proceed), and add several drops of blood. After thoroughly mixing, permit to stand ten to twenty minutes, then centrifugalize for twenty to thirty seconds at a moderate speed. The supernatant fluid is pipetted off until a layer of fluid approximately equal to the depth of the sediment remains. Mix the sediment well with the supernatant liquid, draw up in the pipette and discharge one drop near the end of a perfectly clean glass slide.

Spread the stained drop with the edge of a coverslip square 18 mm. and draw the film to about 6 cm. from the starting point.

The underlying principle of the method involves the Relative Reticulocyte Distribution (R. R. D.) throughout the smear. To know the relative distribution of reticulocytes is important, since it is technically not possible to make a blood film manually so that the distribution of erythrocytes and reticulocytes is equal throughout the preparation. It has been determined, however, that the distribution varies in a definite way when the above technic of preparing smears is followed each time.

The R. R. D. is found as follows: Divide a piece of paper into 10 columns and mark them 1 to 10. Adjust a 4x eyepiece to a tube-length of 190 mm., using an oil immersion objective 1.8 mm. \times 95 N.A. 1.30 in order to obtain the specific microscopic working field. Any other combination giving the same field may be used.

The R. R. D. in the film will usually be found to show about four variations in concentrations as follows: (1) extremely high, (2) high, (3) low, (4) extremely low or absent.

Place the objective on the upper edge of the film near the starting point, that is, the extreme left border of the film. Moving the slide upward in a vertical line, count several

fields that show the highest as well as the moderately high, moderately low and low reticulocyte concentrations. Record the counts of each classification in column 1. When the lower edge of the film is reached, move the slide to the left horizontally 0.5 cm. (measured by the mechanical stage), from this point move the slide downward, count the reticulocyte concentrations as before and record them in column 2. Count ten vertical zones in this manner. If a vertical zone does not show any reticulocyte, mark in the corresponding column a zero.

In a vertical zone 18 mm. in length there will be found approximately 150 microscopic fields, the average field showing about forty erythrocytes. There should be counted and recorded in each column the number of reticulocyte concentrations found in at least eight to ten fields in each vertical 18 mm. zone. Select from the 80 to 100 individual enumerations recorded in the ten columns ten reticulocyte counts distributed as follows:

(a) Two fields showing extremely high concentrations.

(b) Three fields showing high concentrations.

(c) Two fields showing low concentrations.

(d) Three fields showing extremely low to absent concentrations (when selecting the individual reticulocyte counts use as many zeros as there have been zero columns).

Having added the ten reticulocyte counts the total number is referred to as the sum of reticulocytes (S_R). In the same ten fields there are 400 erythrocytes. The ratio of reticulocytes to erythrocytes is therefore S_R

The percentage of reticulocytes is $\frac{S_R}{400}$

$\frac{400}{400} \times 100$

or per cent = $S_R \times .25$

TUBERCULOSIS, Sanocrysin Treatment, Henrichsen, K. J., Sweany, H. C., and Hruby, A. J. Am. Rev. Tuberc. (Suppl.) 28: 1, 1933.

About 50 per cent of tuberculous patients having a stationary or downward progressive advanced tuberculosis of the "B" or "C" types, when given Sanocrysin in well-regulated doses, will show (1) a rather prompt cessation of symptoms (drop of temperature and pulse, increase of weight, and improvement of general condition), which changes tend to be permanent if the patient observes the usual measures of hygiene; (2) a clearing of tuberculous infiltration, with marked fibrosis and contraction of cavities, as shown by roentgenograms; and (3) changes from unfavorable to favorable laboratory findings.

The remainder of the treated patients may be divided about equally into three classes: those who show a temporary improvement for a few weeks to a few months; those who show no favorable change; and those who will not tolerate the drug at all.

By all the methods of control available and carefully analyzed figures, we can conclude that there is a shortening of the convalescent period or prolongation of life or both in the treated patients of Group 1.

There seems to be a real indication for the drug's use as a supportive treatment in collapse therapy when patients are not doing well, and when there is a beginning spread to the other side, or when there is need to clear up a slight involvement of one side in preparation for collapse therapy on the other.

Sanocrysin may be used to speed the convalescence of patients with favorable prognosis, but this is usually unnecessary.

The drug may be added to the armamentarium of the tuberculosis specialist for use when other measures are unavailable, as well as for other "reacting" types when the skill of the physician is able to direct a successful course.

More experiment is warranted, so that indications, or contraindications, and more favorable types may be better established, in all ages, stages, complications, types of the disease, and in both sexes.

AGGLUTININS: The Agglutinin Content of the Blood Following Typhoid and Paratyphoid Immunization. Foord, A. G., and Forsyth, A. *Am. J. Clin. Path.* 3: 333, 1933.

Agglutination tests conducted simultaneously with the same flask of antigen on serums of 120 nurses and internes immunized by three weekly subcutaneous injections of triple typhoid paratyphoid A and B vaccine furnished by the New York State Department of Health revealed the presence of typhoid agglutinins in a dilution averaging 1:320 to 1:640 at the end of twenty-three days following the last injection.

Tests at longer intervals of several months and to from five to nine years demonstrated persistence of agglutinins in all the serums studied in lower, but clinically significant amount; for example, 1:160 after six and nine years, respectively.

Agglutinins against paratyphoid bacilli were demonstrated at titers averaging somewhat lower than the corresponding titer against the typhoid bacillus.

In evaluating a positive agglutination test against typhoid or paratyphoid organisms, the history of prophylactic immunization must be considered, even though many years have elapsed since the inoculations.

PREGNANCY: The Rabbit Ovulation Test, Anklesaria, S. B. *J. Univ. of Bombay* 1: 108. 1933.

All the biologic tests of pregnancy depend on the presence, in the body, of living chorionic tissue in biologic contact with the blood stream.

Pregnancy is a physiologic condition in which chorionic tissue is present and is in contact with the blood stream; but should a man or woman be the unfortunate possessor of a tumor containing chorionic tissue, i.e., a chorioneplithelioma, he or she should give a positive biological test. Thus its use in the diagnosis of these tumors both in the female and in the male in the testis for example, is quite apparent.

In normal pregnancy the test is positive as early as three weeks after conception. It becomes negative in two to seven days after puerperium, the rabbit ovulation test becoming negative earlier (forty-eight hours).

In abnormal pregnancy the tests are useful in the diagnosis of ectopic gestation, abortions in various phases, and hydatidiform mole.

In ectopic gestation the test remains positive so long as there is a certain amount of living chorionic tissue in biologic contact with the maternal tissues. The author found it repeatedly useful and in only one case out of seven was it negative; this case being of long standing with no living chorionic tissue. In such cases, however, the danger of ectopic gestation is much less. The author shall, however, emphasize the fact that a full dose of 24 c.c. of urine and a forty-eight-hour interval between the first injection and the laparotomy will go a great way in diminishing the number of negative results.

In cases of death of the ovum in uterine pregnancy, the reaction takes some time to become negative, the time being greater in advanced gestation; it may take as long as a month or more. The significance of a positive reaction becoming negative is great in diagnosing death of the ovum.

Though the reaction disappears within forty-eight hours or a little more in the normal puerperium, it takes two months or a little more to disappear after the passage of a hydatid mole, though the mole may not be malignant.

In cases of hydatid mole and chorioneplithelioma, the author has found the test positive with very small amounts. He is determining at present the smallest quantity of urine that would give a positive reaction in normal pregnancy, which work will be published in a future paper.

TUBERCLE BACILLI, Heavy Drop Method for Demonstration of, in Sputum, Kornetov, I. *Klin. Med. Moscow* 11: 109, 1933.

A particle of the suspected sputum is placed in the center of the glass slide. The amount is determined by its consistency. If the sputum is fluid, a particle about 10 mm. in diameter may be used; if it is thick, a particle of a diameter not exceeding 5 mm. is

used. Five drops of a mixture of 20 c.c. of 10 per cent ammonium hydroxide solution and 30 c.c. of 96 per cent alcohol are added. By means of a thin glass rod the sputum and solutions are mixed. In a few minutes five more drops of the mixture are added as before. If a homogeneous mass has not been obtained, five more drops are added. After five minutes the mass is usually homogeneous. The mixture is then dried in an autoclave or an incubator. The preparation dries in the air in two to three hours, under proper temperature in about twenty minutes. Excessive heating should be avoided. The dried mass is stained without fixation. Sufficient carbolfuchsin to cover the mass is added. The slide is heated until it steams; this procedure is repeated twice and the carbolfuchsin is decanted. The preparation is treated repeatedly with 5 per cent nitric or sulphuric acid, the acid being washed off each time, so that finally the preparation assumes a slight rosy color. The preparation is then treated with a few drops of a 1 to 500 solution of methylene blue or a few drops of a 1 to 500 solution of chrysoidin. The slide is washed and dried.

By this method each field contains from 6 to 20 times more tubercle bacilli, if such are present, than the usual smear.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The History and Epidemiology of Syphilis*

THIS book is a series of three lectures delivered under the Adolph Gehrman endowment at the University of Illinois College of Medicine in March, 1933. The first lecture discusses the origin of the disease, presenting the evidence for and against its importation into Europe by the sailors of Columbus. The second lecture summarizes the historical development of our understanding of the nature of the disease and its treatment. The third discusses present epidemiologic problems and outlines preventive possibilities. For further discussion see editorial in this number.

The History of Dermatology†

IT IS only within the last hundred years that dermatology has become a specialty. As the author remarks, the history of dermatology, especially prior to 1800, is the history of medicine in general. He has, however, abstracted from the history of medicine the points that are of particular interest in connection with dermatology and the first sixty odd pages are devoted to the facts of dermatology as they have been developed in the history of medicine up to the beginning of the nineteenth century. The second two-thirds of the book deals with the historical development of dermatology as a specialty. This has been done in a very readable and informative way. Sometimes the reader wishes the author had more space to devote to the development of the personalities of the men who contributed so much to the specialty.

A distinct innovation, as an appendix, is the author's historical index on dermatology. In it he lists the various dermatologic diagnoses alphabetically, with brief summaries describing when such diseases were first mentioned in history, with other outstanding historical points about them.

*The History and Epidemiology of Syphilis. The Gehrman Lectures, University of Illinois, 1933. By Wm. Allen Pusey, A.M., M.D., LL.D., Professor of Dermatology Emeritus, University of Illinois, Sometime President of The American Dermatological Association and of The American Medical Association. Cloth, pages 113. Springfield, Ill. Charles C. Thomas, 1933.

†The History of Dermatology. By Wm. Allen Pusey, A.M., M.D., LL.D., Professor of Dermatology Emeritus, University of Illinois; Sometime President of The American Dermatological Association and of The American Medical Association. Illustrated, cloth, pages 223. Springfield, Ill. Charles S. Thomas, 1933.

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EDITORIAL

The Origin of Syphilis

TWENTY years ago it was generally understood that, as far as the civilized world is concerned, syphilis is one of the few new diseases that have been introduced since ancient times. It was understood that the disease was contracted by the sailors of Columbus from the Indians of the New World, carried back into Spain and Portugal from where it rapidly spread to the Spanish soldiers who, as mercenaries, introduced it into the armies of Charles VIII of France and by which it was carried into Italy as far as Naples. The army, victorious against the Neapolitans and others in their pathway succumbed to the disease, retreated into France and there was disbanded, to send syphilis home to all of the countries of Europe which had contributed to the mighty army of Charles. The Italians called it the Spanish disease, the French called it the Italian disease, the English, Germans and Turks called it the French disease and the Russians called it the Polish disease. The Spanish, alone, called it the disease of Haiti.

Aside from the time relationship, epidemiologic considerations pointed to an infection of a previously uninvaded multitude of hosts whose lack of acquired immunity was chiefly responsible for the extreme virulence of the epidemic.

But in 1913 Karl Sudhoff presented his evidence that syphilis had existed in Europe prior to Columbus' discovery of America. This opened the question anew with the result that some evidence was collected through several years purporting to show that syphilis was an ancient disease in Europe, even that the ancient Egyptians had suffered from syphilitic osteitis, as evidenced by lesions found in mummies. Probably it is well that the question was raised by an authority such as Sudhoff, since this necessitated a reexamination of the records and has resulted in the discovery of additional facts far more convincing than those previously available. The evidence for and against importation from the New World has been collected and presented by Pusey in a most interesting manner in his Gehrman Lectures before the University of Illinois.¹

In Germany in 1495 syphilis was often mentioned as the "never-before-seen-and-heard-of-disease." The earliest known German state document on syphilis was published in 1495, well after the time of Columbus' return and the introduction of syphilis into Germany.

Oviedo, who was in Barcelona at the time of Columbus' return and knew him and members of his crew, described how the disease was contracted from Indian women by Spaniards with Columbus and how it was brought by them to Spain. Las Casas, whose father accompanied Columbus on his second voyage, and who, himself, lived in Haiti for many years remarked "there were and still are two things which at the beginning were very dangerous to the Spaniards. One was the disease syphilis, which in Italy is known as the French malady." He, himself, describes seeing the first cases in Seville among Columbus' sailors. "It is a thing well verified that all the incontinent Spaniards that did not have the virtue of chastity on this island were contaminated."

Dias de Isla, who was a physician at Lisbon at the time of Columbus' return, described the new disease as it occurred among Columbus' sailors and later in the general population. The first and second editions of his work have been discovered and what is more interesting, the original manuscript. The original manuscript contains certain paragraphs on syphilis which were omitted from the printed work. This may have been for patriotic reasons. Dias de Isla, practicing in Barcelona in 1493, saw the very entrance of syphilis into the Old World. He states definitely that the disease was unknown before the year 1493, that it was brought by the crew of Columbus on their return from their first voyage, that a majority of the crew were infected, and that he himself treated a number of the sailors. "At the time that Admiral don Xristoual Colon arrived in Spain the Catholic Sovereigns were in the city of Barcelona. And when they went to give an account of their voyage and of what they had discovered, immediately the city began to be infected and the aforesaid disease spread."

It has been claimed that bone lesions have been found indicative of syphilis in human beings who died in the Old World long before the discovery of

America. Among others, Williams of Buffalo has made extensive investigations into this. The only incontrovertibly characteristic bone lesion is in the flat bones of the skull. Lesions of long bones might be due to other diseases. No incontrovertible evidence of syphilis of the bone has ever been presented in any skull prior to the time of Columbus except in the New World, where such evidence is relatively abundant. The search therefor has not been haphazard, as is indicated by the report of Elliot Smith based upon the examination of the mummies of 30,000 Egyptians and Nubians.

There is no definite evidence, either from morphologic studies, from historical documents, from medical treatises, or from legal enactments, of the presence of syphilis in Europe prior to the discovery of America. And, as Pusey pointedly remarks, there is no evidence in the prurient literature of the ancients. What fun Boccaccio or the authors of the Arabian Nights would have had in describing the wages of sin did they know of the ravages of syphilis!

But, after the return of Columbus it required but a short time for the disease to spread throughout the known world. The Portuguese explorers lost no time. In India the disease was recognized in 1498, after the arrival of Vasco de Gama who had left Portugal one year earlier. It appeared in Canton, China, in 1505. In Japan it was first described in 1569 when it was imported either by Chinese or Portuguese sailors.

The discovery of a New World gave birth to many new and perplexing problems, not the least among which was that of syphilis.

REFERENCE

1. Pusey, Wm. Allen: *The History and Epidemiology of Syphilis*, Springfield, Ill., 1933, Arthur C. Thomas.

—W. T. V.

CORRESPONDENCE

Remarks supplementary to editorial on "Bilirubinemia and Pneumonia" by R. A. K. in
Journal of Laboratory and Clinical Medicine 18: 1197, 1933

To the Editor:

In order to establish a clear understanding of the significance of the jaundice of lobar pneumonia as it may be seen in its present stage of analysis, the following summary is submitted. This summary represents but a single phase of a concept-totality with a number of ramifications involving jaundice as a whole and its mechanism in any other clinical entity with which it may be associated. Here, however, we are concerned specifically only with the events occurring when jaundice is associated with a disease caused by a bile-soluble organism, the pneumococcus.

1. Icterus, manifest in one or all of three tests performed daily during the acute course of lobar pneumonia, has been found to be practically always present. These tests consist of the icterus index, the aqueous (direct) van den Bergh reaction, and the quantitative serum bilirubin determination, based on van den Bergh's application of Ehrlich's diazo reaction as recommended by Pröscher.

2. The visibility of icterus in the skin or sclerae is of no significance other than that visible icterus is quite characteristic of lobar pneumonia, its detection being largely a question of the visual acuity of the observer. The fundamental data must be obtained from studies on the blood serum. Numerous observations have demonstrated that no threshold for the visibility of icterus can be postulated, since visibility is dependent on qualitative as well as quantitative factors in jaundice.

3. If, regardless of other methods for characterizing the course of lobar pneumonia, cases are classified on the basis of the presence or absence of pleural fluid (not fibrin alone), demonstrable by x-ray, daily physical examination, or, preferably, chest tap, then the following conclusions are admissible:

a. Approximately 70 per cent of hospitalized patients with lobar pneumonia in this series developed pleural fluid, and in this group the mortality was approximately 15 per cent.

b. In the remaining 30 per cent of these patients fluid was not demonstrable in any way ("dry chest" type), and in this group the mortality lay between 75 per cent and 80 per cent.

A figure for the incidence of pleural fluid in pneumonia, closely approximating this finding, is reported by G. Andral, *Clinique médicale, Paris, Gabon et Cie., 1824, 2, 242*, and by R. T. H. Laennec, *Traité de l'auscultation, Paris, G. S. Chaudé, ed. 2, 1826*. It is on the basis of the presence or absence of this fluid that the interpretation of the changes in the jaundice of lobar pneumonia is made, so far as they are related to the outcome.

4. In the white race, in the absence of pleural fluid, the mortality has thus far been 100 per cent unless the icterus index exceeded 16.6. Most white patients whose icterus index curves exceeded 16.6 exhibited the downward turn in the zone 18.7 to 30, and thus far none have died whose maximum index lay in this zone whether fluid was present or absent. When, as occasionally happened, the icterus index of a white patient exceeded 30, recovery then took place only if the icterus index 100 was attained. In the negro, on the other hand, in the absence of fluid, the mortality was 100 per cent unless the icterus index exceeded 100. This is why apparently white patients are said to show negroid reactions if their icterus index curves exceed 30. True crisis is regarded as the favorable termination of the disease when fluid is absent. It is rare.

5. Upon the development of pleural fluid the rising icterus index curve is broken, and the curve tends to fall back to normal. Frequently a patient is seen for the first time after this has already occurred, and the icterus index is found to be normal. Jaundice is then

demonstrable only by the van den Bergh reaction, or in an elevation of the quantitative serum bilirubin. These patients die with direct positive van den Bergh reactions, and resolution is not assured until the van den Bergh reaction (by the ring test) becomes negative. In all cases in which data were available to show that, in the presence of fluid, the icterus index curve turned in the zone 11 to 15, thus far there have been no fatal terminations in the white race.

6. The icterus index 16.6 warrants recognition as the "Death Index" in the white race in the absence of fluid, for it must be passed if recovery is to be expected. It may be regarded as a biologic constant in the white race, and of no significance in the negro. The data suggest further that the icterus index 10, apparently of significance in the white race in conjunction with the formation of pleural fluid, is worthy of careful consideration as another possible constant.

In this entire study of icterus, as it occurs in pneumonia and other conditions, it has become apparent that many commonly accepted theories on which classifications of jaundice are based are not acceptable as working hypotheses, hence it is advisable not to attempt to classify the icterus of lobar pneumonia at the present time.

The study of the pneumonic icterus, as well as all icterus, is extraordinarily fascinating, but like all research, requires that the tests be performed by the research worker himself rather than being entrusted to a technician, unless the technician has time and interest to devote to the work specifically. It is further advisable that a single individual conduct the tests, so that the factor of error will be a constant.

In pneumonia, induced by a specific organism, it does not follow that only pneumococcus pneumonias exhibit icterus. It does follow, however, that in pneumonias caused by Friedländer's bacillus, staphylococci, and the hemolytic streptococcus, the interpretation of the movements of the icterus is wholly invalidated, for such organisms are not bile soluble. Blood cultures, x-ray examinations of the chest, sputum cultures, chest and lung punctures and cultures, and autopsies have on several occasions disproved apparent exceptions to the findings described above, especially when erroneous clinical diagnoses were made, or when the invading organism was other than the pneumococcus.

Pleural fluid is generally most conspicuous in patients approaching a fatal outcome in its presence, frequently being very transient in those that recover uneventfully, but is readily demonstrable by timely chest-tap. It may be differentiated from true empyema in that, although it is a cellular fluid, the supernatant is bright yellow as contrasted with the more or less colorless supernatants of empyema, and its cells, as exhibited by supravital staining technic, comprise a relatively high percentage of living macrophages, in contrast with the dead polymorphonuclears of empyema.

NORMAN W. ELTON, M.D.

READING, PA, SEPTEMBER 5, 1933.

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CLINICAL AND EXPERIMENTAL

THE MACROPOLYCYTE*

W. E. COOKE, M.D., WIGAN, ENGLAND

THE statement that megakaryocytes are occasionally found in the peripheral blood has been repeated in textbooks and monographs during the past thirty years, but I have never seen in blood films a cell which faithfully resembled the multinucleated giant cell of the marrow; nor have I seen photographs or drawings of the cells so described that are at all convincing.

There is every probability that these so-called megakaryocytes belong to one or other class of macropolycytes.

DEFINITION

The normal neutrophile polymorphonuclear leucocyte, for brevity called the "polymorph," measures, in fixed and stained films 10 to 12 and occasionally up to 14 micra in diameter. In certain conditions giant polymorphs measuring 16 to 20 or more micra in diameter are found, to which the descriptive title "Macropolycyte" is given (1927).

Morphologically there are three types of macropolycytes. The first or Type 1 resembles the other polymorphs in the blood film in that its nuclear structure and fine oxyphil cytoplasmic granules are indistinguishable from those in the polymorph. The only differences from that cell are the large size and frequent hypersegmentation of the nucleus.

The Type I macropolycyte is found: (1) in health; (2) in infections and intoxications where there is bonemarrow reaction, e.g., the acute pyrogenic

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infections, tuberculosis, malaria, the exanthemas, carcinoma, myeloid leucemia, and in rabbits with experimental anemia due to ricin or repeated hemorrhages; (3) pernicious anemia.

The Type I macropolyocyte is illustrated in Plate II, Figs. 2 to 7, and in Plate III, Figs. 9, 10, 11, 12, 13, and 14.

The Type II macropolyocyte resembles the megakaryocyte of the marrow in that its nucleus is gnarled and somewhat condensed and may be of the simple horseshoe type, or may have as many as 14 segments. The cytoplasmic granules are coarse and are not all oxyphil as in the polymorph, but azurophil granules are present in varying numbers.

The cell is found in pernicious anemia and is illustrated in Plate III, Figs. 15 to 25.

The third type of macropolyocyte has features of both the preceding types. It resembles the Type I cell in having fine oxyphil granules in the cytoplasm, while the general nuclear conformation is that of Type II. It differs from both in nuclear structure. As the figures on Plate IV show, the nuclear structure is a very open meshwork suggesting a deficiency in basichromatin, and the bulk of the nucleus appears to be large in comparison to the cytoplasm. The nucleus is often rolled and folded on itself, but hypersegmentation is not frequently seen. This type like Type II occurs in pernicious anemia.

THE POLYMORPH IN HEALTH AND IN INFECTIONS

For the better appreciation of the macropolyocyte a few remarks on the polymorph are necessary. The polymorph enters the blood stream from the marrow with a single-lobed nucleus, and as its life in the circulation continues, the nucleus becomes more lobulated until toward the end of its existence it has four or five segments united by fine strands of chromatin (Plate I). In the blood stream, therefore, there are, normally, cells representing the various periods from youth to senility, and it was found by Arneth (1904) that the numbers of polymorphs with one, two, three, four, or five nuclear segments were remarkably constant in health and could be classified on those lines. Our average normal count, that is the percentage of cells in the various classes corresponding to nuclear segments, is:

Class	I	II	III	IV	V
	10	25	47	16	2

This was the average of the counts of 90 normal persons of both sexes between the ages of twelve and fifty-five years. The greatest possible care was taken to exclude infection at the time the blood was taken, and for two months previously. The teeth, throat, nose, ears, chest, abdomen, and urine were examined, and in many cases radiograms were taken of the chest and teeth.

The length of life of the polymorph in the blood stream has been estimated on experimental and clinical grounds (Ponder, 1926) (Cooke and Ponder, 1927) to be approximately three weeks. The figures given above represent a polymorph population comparable to infants, children, men, old men, and senile men, in which the deaths in the older groups are exactly compensated for by the births from the marrow. The figures of the polynuclear count tell us

whenever this normal state becomes altered, whether by the addition of an unusually large number of young cells, by the removal of an unusually large number of old cells, by both these conditions combined, or by some interference with the normal development.

It will be noted that no class is provided for cells with more than five nuclear segments. Such polymorphs are rare in normal blood and when they do occur they are placed in Class V.

In infective conditions the percentage of cells of Classes I and II is increased, and the older cells of Classes IV and V rapidly disappear from the circulation. The count has, therefore, a left-handed drift, as in the following examples:

	I	II	III	IV	V
Normal	10	25	47	16	2
Typhoid fever	54	31	15	—	—
Scarlet fever	44	43	13	—	—
Measles	45	39	16	—	—
Erysipelas	45	40	14	1	1
Diphtheria	41	39	17	3	—
Rubella	30	35	32	3	—
Varicella	33	35	28	4	—
Pertussis	34	39	27	—	—
Puerperal sepsis	42	37	21	—	—
	I	II	III	IV	V
Cerebrospinal fever	40	45	14	1	—
Lobar pneumonia	57	26	15	2	—

These facts make the appearance of the macropolyocyte the more remarkable, because, although a fairly rare cell, it is found in infections frequently associated with a polymorphic leucocytosis and always with a left-handed polynuclear count.

Before proceeding to further discussion, the moment is opportune to suggest that, although the Type I macropolyocyte found in pernicious anemia may be morphologically identical with its prototype found in health and in intoxications, the cause of its appearance is probably very different. For this reason the Type I macropolyocytes of health and of intoxications would be better considered together, and the types found in pernicious anemia classed under the heading "the macropolyocytes of pernicious anemia."

THE TYPE I MACROPOLYCYTE IN HEALTH AND IN CONDITIONS OF ABNORMAL MARROW ACTIVITY

1. *In Health*.—Macropolyocytes are extremely rare in health, no example being seen in the blood films of the 90 investigated normal persons that formed the basis of our average normal count. Recently, however, I observed a macropolyocyte in a man, aged thirty-four years, whose polynuclear count had previously been included in the original 90. He was reinvestigated, but no focus of infection could be found. The counts of the two occasions were almost identical:

I	II	III	IV	V
11	31	42	14	2

The count has a weighted mean of 2.65, which falls close to the average mean of 2.74, and within the limits of normality. I think we must accept the fact that macropolycytes do very occasionally appear in normal blood films.

2. *In Conditions of Abnormal Marrow Activity.*—This type is illustrated by the Figs. 2 to 7 in Plate II.

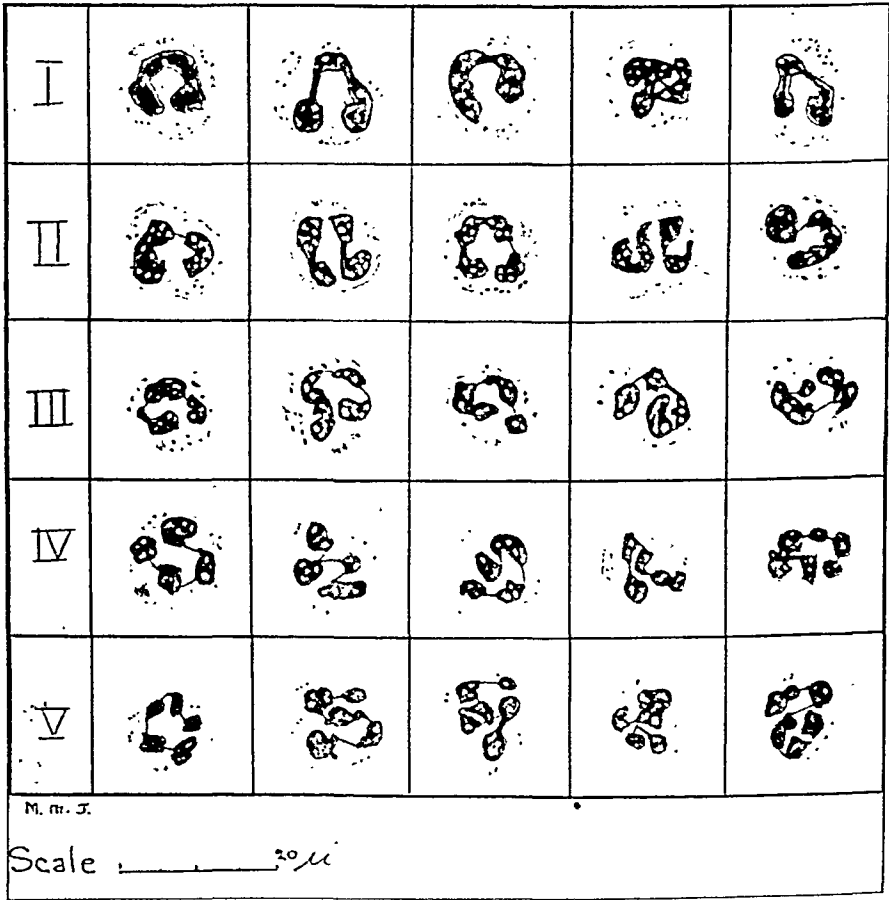


PLATE I.

The five classes of the polynuclear count. If there is any band of nuclear tissue except a chromatin filament, uniting the nuclear masses, those parts must not be considered as separate segments. Class I, No. 2, Class II, Nos. 3 and 4, Class III, No. 4, Class IV, No. 5, illustrate this point. In Class V, No. 4, the upper segment of the nucleus overlies the segment below it, but the thin chromatin filament uniting them can be seen on the left.

Plate II. Fig. 2 is a macropolycyte found in a case of staphylococcal osteomyelitis, which had the following blood count:

Total leucocytes	18,000 per c.mm.
Polymorphs	87 per cent.
Polynuclear count	41, 43, 15, 1, 0.

Plate II. Fig. 3 is a macropolycyte from a case of streptococcal septicemia.

Total leucocytes	26,000 per c.mm.
Polymorphs	79 per cent.
Polynuclear count	34, 43, 21, 2, 0.

Plate II. Figs. 4 and 5 are macropolycytes from a case of mixed infection by *Staphylococcus aureus* and *Bacillus coli*.

Total leucocytes	23,700 per c.mm.
Polymorphs	88 per cent.
Polynuclear count	45, 39, 15, 1, 0.

Plate II. Fig. 6 shows a macropolycyte from a case of streptococcal endocarditis.

Total leucocytes	16,000 per c.mm.
Polymorphs	76 per cent.
Polynuclear count	39, 34, 27, 0, 0.

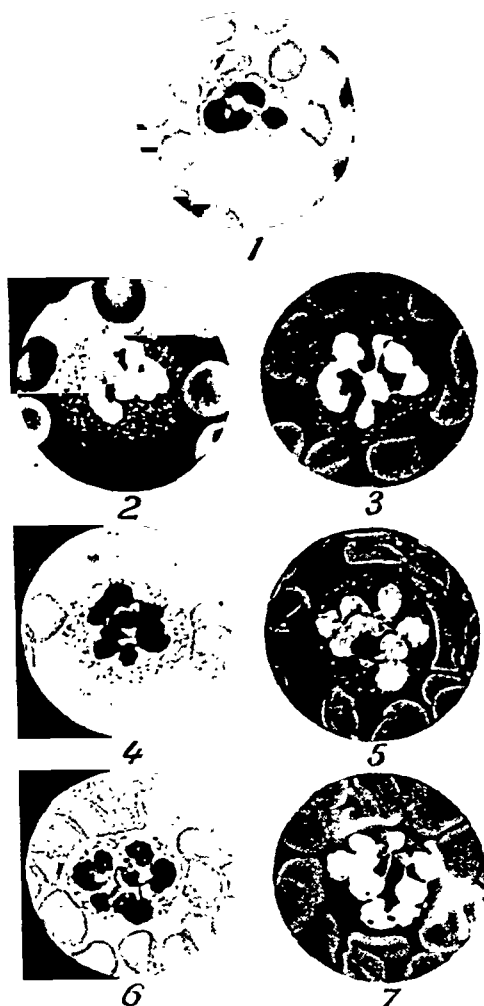


PLATE II.

Fig. 1.—A normal polymorph.

Fig. 2.—Macropolycyte, Type I, from a case of staphylococcal osteomyelitis.

Fig. 3.—Macropolycyte, Type I, from a case of streptococcal septicemia.

Figs. 4 and 5.—Macropolycytes, Type I, from a case of mixed infection by *Staphylococcus aureus* and *Bacillus coli*.

Fig. 6.—Macropolycyte, Type I, from a case of streptococcal endocarditis.

Fig. 7.—Macropolycyte, Type I, from a case of carcinoma of the breast.

($\times 1,000$ diameter.)

Plate II. Fig. 7 is a macropolyocyte from a case of carcinoma of the breast.

Total leucocytes	14,500 per c.mm.
Polymorphs	76 per cent.
Polynuclear count	35, 36, 26, 2, 0.

It will be seen that the macropolyocytes have hypersegmented nuclei, although polymorphs with single and bilobed nuclei predominate in the blood films, with, in many cases, an occasional myelocyte. What is the explanation of their presence? It is not easy nor particularly important to determine why macropolyocytes appear in health and in infections, because they are rare and do not seem to have any prognostic or diagnostic significance. There are two possible explanations of their presence. The first that they represent an occasional polymorph that has escaped the physiologic mechanism whose function is the destruction of aged leucocytes and their removal from the blood stream. A polymorph has been imprisoned in some tissue space, exceeded its allotted span of life, and continued to develop beyond the normal limits. The result is that it has become a giant. The second explanation is that they are cells whose development has been altered by an abnormal medium to which they have been exposed in the marrow during the first period of their development.

THE MACROPOLYCYTES OF PERNICIOUS ANEMIA

1. The Type I macropolyocyte is frequently found in pernicious anemia, and here again it is necessary to consider the general behavior of the polymorph in this disease. Small forms, micropolyocytes, are often encountered (Fig. 1, Plate III). These cells measure 10 microns or less in diameter, and the nucleus may be single or multilobed. From the micropolyocyte all gradations occur up to the macropolyocyte. As we have seen above in the infective state the polynuclear count is left-handed. Many uncomplicated cases of pernicious anemia, however, show the remarkable state of having an increase in the cells of Classes IV and V, and the count appears to be actually right-handed, as the following figures show:

	I	II	III	IV	V
Normal	10	25	47	16	2
Case 1	8	20	39	22	11
Case 2	6	27	40	22	5
Case 3	7	20	44	24	5
Case 4	7	16	29	39	9
Case 5	20	31	17	10	10
Case 6	16	22	32	16	14
Case 7	10	15	38	24	13
Case 8	12	34	26	20	8
Case 9	6	20	42	20	12
Case 10	8	24	30	26	12
Case 11	6	18	36	24	16
Case 12	6	20	40	26	8
Case 13	14	28	23	20	15
Case 14	12	28	36	18	6

This condition is not seen in any microbial disease.

It has been pointed out above that no class is made in the polynuclear count for cells with more than five lobes in their nuclei, because cells with more



PLATE III.

Fig. 1.—A polymorph of small size, a micropolycyte.

Figs. 2, 3, 4, 5, 6, 7, and 8.—Polymorphs of normal size showing hypersegmentation of the nucleus.

Figs. 9, 10, 11, 12, 13, and 14.—Macropolycytes, of Type I.

Figs. 15 to 25.—Macropolycytes, of Type II.

($\times 1,000$ diameters.)

than five lobes are rare in normal blood, and if any are found they are placed in Class V. In pernicious anemia, on the contrary, hypersegmentation of the nucleus in cells of normal size is frequent. The nucleus may have six, seven, eight, or even ten or more divisions. Plate III, Figs. 2 to 8 are examples of this

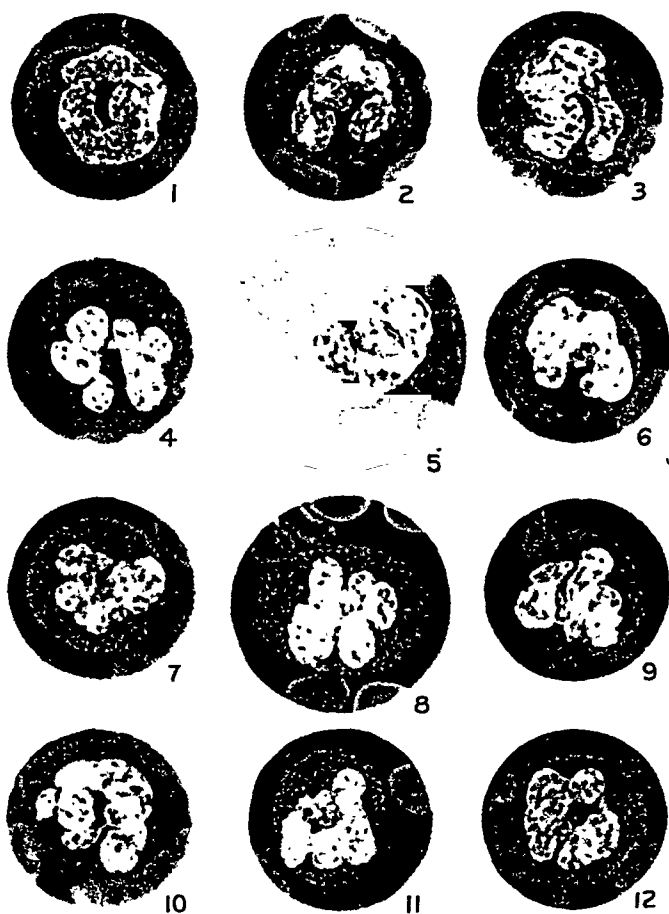


PLATE IV.

Figs. 1 to 12 illustrate the third type of macropolycyte. The figures show the large bulk of the nucleus, the structure of which appears to be a more open meshwork than normal, suggesting deficiency in basichromatin. The fine cytoplasmic oxyphil granules are also seen. ($\times 1,000$ diameters.)

nuclear hypersegmentation in cells of approximately normal diameter. Plate III, Figs. 9 to 14 are examples of the Type I macropolycyte. Its frequency in pernicious anemia contrasts very sharply with its rarity in health and in infective conditions, and the cause of its appearance is probably not the same.

There are several possible causative factors. Polymorphs of normal size with hypersegmented nuclei accompany the Type I macropolycyte in this disease, and the first thoughts are that there is some biochemical change in the plasma that prematurely ages the polymorph and alters its life history; the second, that the mechanism for the elimination of old polymorphs is in abeyance. The third reasonable hypothesis may be advanced that the polymorph is inherently abnormal owing to a defect in its parent, the hemocytoblast, or in its environment. Possibly, too there is a single biochemical defect that is responsible for all three conditions. The liver diet, or hog's stomach alters the polymorph picture in a few days. Macropolycytes and polymorphs with hypersegmented nuclei disappear, and at the same time the morphologically and probably also chemically abnormal cells of the hemoglobiniferous series are replaced by cells of normal appearance.

2. The Type II macropolycyte is found only in pernicious anemia, and appears in blood films during serious relapses. Almost all the cases in which it has been found have had erythrocyte counts of less than 1,000,000 per c.mm., and before the era of liver treatment all died within a short time of its appearance. After death these large cells are found in the liver and preaortic glands in association with islets of megaloblasts. Plate III, Figs. 15 to 25, illustrates the variations in this type of cell. The cells are large, measuring up to 24 or more microns in diameter, and resemble the megakaryocyte of the marrow.

3. The third type of macropolycyte, illustrated on Plate IV, is also found in pernicious anemia only. Its characteristics have already been described and are seen in the figures. Like the second type it is found in grave relapses and its appearance before the introduction of modern treatment was of grave prognostic import. We believe this cell is a variant of the preceding type and the explanation of its presence in the blood stream to be the same.

These cells do not resemble any embryonic or postnatal blood stream cell, nor are they accurate replicas of the megakaryocyte of the marrow. The life history would appear similar to that of the polymorph in that they seem to commence circulatory existence with a single-lobed nucleus which later becomes segmented. The length of life we do not know, nor have we any means of calculating it. There is considerable doubt about their origin. They may be an expression of the abnormal reversion to embryonic type of the marrow hemocytoblast as are the hemoglobiniferous cells in pernicious anemia. On the other hand, the cells may not arise in the marrow, but originate in the mesenchyme descendants in the liver and hemolymph glands that have undergone abnormal metaplasia.

CONCLUSIONS

1. The macropolycyte found in health and in infections has no diagnostic nor prognostic significance, and is probably due to the accidental prolongation of the lifetime of a neutrophile polymorphonuclear leucocyte.

2. The causation of the appearance of the Type I macropolycyte in pernicious anemia is closely related with the factor responsible for the production

of hypersegmentation in the nuclei of the polymorph. Whether the factor is a biochemical change in the environment or an inherent defect in its parent cell is not known.

3. The Types II and III macropolyocytes may have their origin in the marrow, or may arise from the endothelium of the liver and hemolymph glands. In the first case they are products of abnormal reversion of the hemopoietic tissue, and in the second of abnormal myeloid metaplasia.

Photomicrographs by C. F. Hill and W. E. Cooke.

I most gratefully acknowledge the kind permission of Messrs. Charles Griffin and Co., London, to reproduce Plate I from the Polynuclear Count, Dr. Paul Chevalier, for Plate II from Le Sang, and the Editors of the Journal of the Royal Microscopical Society for Plates III and IV.

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SODIUM RICINOLEATE*†

I. AN ATTEMPT TO DETERMINE ITS ACTION IN THE ALIMENTARY TRACT

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SODIUM ricinoleate, one of the soaps of castor oil, has been and is at the present time, widely used clinically. It appeared to us that an attempt would be desirable to determine the site of its action and the mechanism of its action when used in the intestinal tract.

Two methods were employed in the attempt to elucidate these points. The first was by means of a dye, Sudan IV or azo-orthotoluene-azo-B-naphthol. This dye is soluble in oils, ethers, chloroform, fat, fatty acids, and soaps. It is insoluble in aqueous solutions. Sudan IV was first used by Daddi in 1896 in a study of fat absorption. When a fatty acid in which Sudan IV has been dissolved is saponified, the Sudan IV remains with the fatty acid and the result is a pink soap. Also, if the dye is mixed with the soap and hydrochloric acid added, the Sudan IV will remain with the fatty acid radical. In view of this property of Sudan IV, it would seem to be an acceptable guide as to the route taken by the fatty acid radical during digestion. It was found by Mendel and Daniels (1912) that Sudan IV fed alone caused no staining of the fat depots, hence was not absorbed. This was checked by Gage and Fish

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(1924) who found that animals fed boiled rice and cooked egg white with which Sudan IV was intimately mixed showed no absorption which they could detect.

The second method of attack was discovered by Edmunds in 1877 and put to use by S. H. Gage in 1924 in his study on the absorption of fats. In this method the minute fat particles (chylomicrons) in the blood were counted. Edmunds said, "If fresh, transparent blood serum with a few corpuscles shaken out of the edge of the clot be viewed, the serum is seen filled with a nebulous haze of points as is mote laden air in a sunbeam or in the electric light." These small particles were counted by means of a net micrometer in the eyepiece of the microscope and a dark-field illuminator. The particles were easily recognized because of their continuous Brownian movement. The technique used was that of Gage and Fish (1924) in which a small drop of blood was placed on a No. 1 cover slip. This was then put on a slide and the excess blood pressed out. The preparation was oiled around the edge of the cover slip to prevent drying.

With these two methods the action of sodium ricinoleate in the intestinal lumen was studied and compared with that of ordinary fats and fatty acids.

The literature on the subject of fat absorption is not in strict accord. Radziejewski (1868) believed that fats were, for the most part, absorbed as soaps. He based this belief on experiments in which he measured the amount of soap fed to animals and the amount excreted in the feces. He believed there was less in the feces, indicating absorption. Plant (1908) isolated loops of small intestine 55 to 75 cm. in length in which he placed fats and soaps. According to his findings, neutral fats were slightly absorbed and soaps in greater quantity. Such a procedure as Plant's would, presumably, alter physiologic relations considerably. Whitehead (1909) believed that fats were absorbed mostly as soaps with probably a small amount taken up as neutral fat. The work of Clark and Clark (1917) indicated that fat could be taken up by the leucocytes and transported to the lymphatics in the unsplit form. Bloor (1916) said, "The way in which food fat (or at least a part of it) gets from the intestine into the blood has been quite satisfactorily determined. It is saponified in the intestine, absorbed in the water soluble form as soaps and glycerol, resynthesized by the intestinal cells and passed into the chyle and thence to the blood as neutral fat (glycerides) suspended in the plasma in a very finely divided condition." Bang (1918) agreed that fats were absorbed as soaps and were changed back to neutral fats in the intestinal wall. Bloor (1922) still believed that fats could be absorbed as soaps but also said that fatty acids and glycerol could be taken up and resynthesized into fats. In 1925 he stated that fatty acids and glycerol were readily absorbed and that in their passage through the intestinal wall they were resynthesized into fats. There was no statement made about the absorption of soaps.

Verzar and Kuthy (1929) advanced another idea as to the method of fat absorption. They showed that oleic, stearic, and palmitic acids would make clear solutions with sodium glycocholate and sodium taurocholate. This would render fatty acids diffusible and so transportable from the intestinal lumen to the blood stream.

Perhaps the most commonly accepted theory at present is that neutral fats enter the small intestine as such, but at this point the reaction becomes alkaline and the lipase breaks the neutral fat into fatty acids and glycerol. The bile aids by breaking the neutral fat into minute particles so that the lipase will have access to the neutral fat more easily. The fat then enters the intestinal wall as fatty acid and glycerol where it is resynthesized into neutral fat, probably by the lipases found in the intestinal cells. At least the fat is in the neutral state by the time the lacteals are reached.

The question still exists as to the possibility of the absorption of fats as soaps. If fats are absorbed as soaps, a soap made from a fatty acid in which Sudan IV is dissolved to identify the fatty acid radical, in case absorption takes place, indicates its eventual deposition by pink fat depots. Also after giving the soap, the chylomicron (fat particle) count should increase, as it does after a fat meal. According to Bloor, Gillette, and James (1927) the chylomicron count, in general, parallels the quantitative determinations of fat in the blood.

In the studies made in this laboratory on the utilization of fats and soaps in the intestinal tract, white rats were used. Whenever possible the animals were not forcibly fed, but in the case of the soaps this was necessary. Here, under ether anesthesia, a small catheter was passed into the stomach and the soaps introduced. Fearing that ether anesthesia might influence absorption, control animals were anesthetized and fed lard. The chylomicron count showed the same increase as in unanesthetized animals. All animals were starved twenty hours before the experiments. There are shown protocols of animals that were fed lard, butter, oleic acid, castor oil and sodium ricinoleate. Soaps were well tolerated when fed by mouth. Intraperitoneal injections of lard, butter, castor oil and sodium ricinoleate were also made in an attempt to determine if absorption from the peritoneum takes place. The following protocols are illustrative of the results obtained.

PROTOCOLS

Rat 1, on June 4, was given ether anesthesia at 9:30 A.M. as a control on its effect in parallel experiments. It was immediately given a meal of toast and lard in which Sudan IV had been dissolved. Chart 1 shows the chylomicron curve during the digestive cycle. The curve is typical and corresponds to those found by Gage and Fish (1924). June 5 and 6 the animal was given stained lard and was chloroformed June 6. The fat of the inguinal, dorsal, omental and kidney regions was stained pink, suggesting good absorption.

Rat 2 was fed soap made from pure lard containing dissolved Sudan IV. A total of 3.1 gm. of soap was fed in 10 per cent solution from May 11 to May 24. The animal remained in good condition throughout and ate corn and oats freely. May 24 the animal was chloroformed. The fat in the depots and in the omentum was not stained. There was some pink material in the stomach. No evidence of absorption was seen.

Rat 3, May 25 at 11:00 A.M. was fed toast spread with butter in which enough Sudan IV had been dissolved to give the butter a distinct red color. This was eaten freely. May 26 at 11:45 A.M. the animal was again fed colored butter on toast and ate freely. At 3:15 P.M. it was chloroformed. The fat of the mesentery, of the other depots, and around the kidneys was decidedly pink in color which indicated good absorption of the butter.

Rat 4 was fed oleic acid stained with Sudan IV. The chylomicron count was made twenty-four hours later and the results are shown in Chart 2. On each of the two following days the animal was fed 1 c.c. of the stained fatty acid. Four hours following the last feeding the animal was chloroformed. The omental and perirenal fat was stained deeply pink. The fat depots of the groin and back were well stained, and there was some pink fat all through the small intestine. Oleic acid was quite irritating and produced some diarrhea, pink material appearing in the stools. Despite this, there is evidence that oleic acid is absorbed.

Rat 5. Oleic acid containing dissolved Sudan IV was saponified and 1 c.c. of an 18 per cent sodium oleate solution fed daily for four consecutive days. The results of the chylomicron count are shown in Chart 3. On two succeeding days the animal was fed 1 c.c. of the soap and chloroformed four hours after the last feeding. None of the fat depots were stained, although an abundance of pink material remained throughout the lumen of the small intestine. The chylomicron count indicates that there was no fat absorption, as does the absence of stained fat.

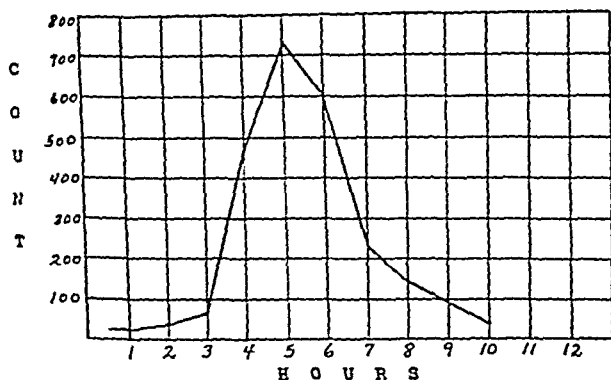


Chart 1.—Rat 1, chylomicron curve.

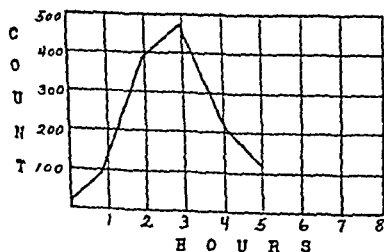


Chart 2.—Rat 4, chylomicron curve.

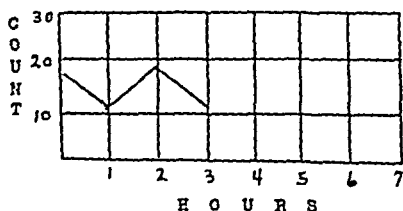


Chart 3.—Rat 5, chylomicron curve.

Rat 6, at 8:00 A.M., was fed toast spread with castor oil in which Sudan IV had been dissolved. Twenty-four hours later it was again fed toast and dyed castor oil and was chloroformed at 1:30 P.M. There was pink material in the stomach and small intestine but none in the colon. No staining of the fat in the mesentery, around the kidneys or in the fat depots in the inguinal or back region was seen, indicating lack of absorption.

Rat 7 was fed 0.23 gm. of sodium ricinoleate in which Sudan IV had been dissolved. The soap was fed as a 10 per cent aqueous solution which formed a clear, transparent pink liquid. The initial dose was given May 9, 1932, and repeated daily until 3 gm. had been fed. May 24 the animal was chloroformed and examined. The fat depots, including the omental fat, were not stained. There was pink material in the stomach and small intestine, but none in the colon. The animal had been given a basic diet of corn and oats and was in good condition at the time of death. There was no evidence of absorption.

Rat 8 was fed 4.3 gm. of sodium ricinoleate in 25 per cent solution from May 21 to May 30, when he died of multiple lung abscesses. There was no staining of the mesentery,

perirenal fat or fat in other depots. The administration of sodium ricinoleate probably had nothing to do with the lung condition.

Rat 9, at 10:00 A.M. was fed 0.2 gm. of sodium ricinoleate by means of a tube, under ether anesthesia. Chart 4 shows the curve of the chylomicron count, the count being plotted on the ordinate and the time on the abscissas. There was no evidence of absorption.

Rat 10 was injected intraperitoneally with 3 c.c. of stained 10 per cent lard soap. Three days later the animal was chloroformed and examined. The perirenal, omental and mesenteric fat appeared pink on the surface, but on cutting there was no penetration of stain into the fat. The fat depot of the dorsal region was unstained. No evidence of absorption was seen.

Rat 11 was injected intraperitoneally with 1 c.c. of butter stained with Sudan IV and the injection repeated the following day. Two days later the animal was chloroformed. There was no staining in any region, including the back, groin, kidney, mesentery, and omentum. The butter and Sudan IV remained in the peritoneal cavity.

Rat 12 was injected intraperitoneally with 1 c.c. of castor oil in which Sudan IV had been dissolved, and this was repeated the following day. Two days later the animal was chloroformed. The material injected still remained in the peritoneal cavity. The omental, perirenal, inguinal and dorsal fat was perfectly white and showed no staining.

Rat 13 was injected intraperitoneally with 3 c.c. of stained 20 per cent sodium ricinoleate and died in three hours. There was no recognizable staining of any body fat. There was

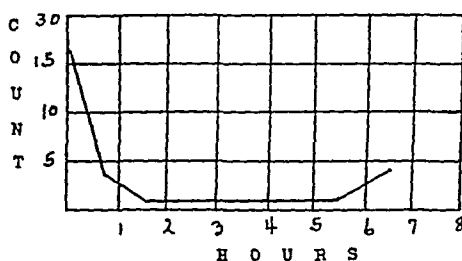


Chart 4.—Rat 9, chylomicron curve.

serous, blood-stained fluid in the peritoneal cavity and the peritoneum was inflamed, but this action is not considered specific for sodium ricinoleate.

There was also noted a very marked increase in the erythrocyte count. We make no explanation of this fact, but did note that 2 c.c. of a 10 per cent silver nitrate solution or 2 c.c. of 2½ per cent nitric acid injected intraperitoneally produced the same results.

Rat 14 at 9:15 A.M., was given 2 c.c. of stained 10 per cent sodium ricinoleate, intraperitoneally. At the time of injection the erythrocyte count was 7,960,000 and at 10:05 the count had risen to 8,200,000. The animal died before another count was made. Examination showed no evidence of fat staining, and there was some fluid in the peritoneal cavity but no more than was injected.

Rat 15, at 10:50 A.M., was injected intraperitoneally with 2 c.c. of stained 5 per cent sodium ricinoleate. Examination showed a small amount of blood in the pleural cavities. There was some soapy material in the peritoneal cavity but no more than was injected, and the peritoneum was inflamed. There was no evidence of staining of the fat in any region. Table I shows the erythrocyte count, white cell count and chylomicron count.

From the above work, based on the study of fat absorption and deposition by the use of Sudan IV and chylomicron counts, it would appear:

1. That the neutral fats, butter, lard, and free oleic acid, are absorbed from the intestinal canal.
2. That the neutral fat, castor oil, is not so absorbed.

3. That soaps made from lard, butter or castor oil (sodium ricinoleate) were not thus absorbed.

4. That none of the above substances were absorbed from the peritoneal cavity.

TABLE I

RAT 15

TIME	ERYTHROCYTE COUNT	WHITE CELL COUNT	CHYLOMICRON COUNT
10:50 A.M.	8,130,000	8,400	90
11:50	9,500,000	5,700	7.5
1:10 P.M.	10,040,000		9
2:20	10,510,000		15
3:00	Animal died		

DISCUSSION AND SUMMARY OF PROTOCOLS

The evidence here presented indicates that soaps are not absorbed from the alimentary tract. This is in direct contradiction to the conclusions of earlier workers who believed that fats are absorbed as soaps, even though a soap splitting enzyme has never been isolated. This work suggests that fats are absorbed as fatty acids and glycerol. We cannot explain the lack of absorption of castor oil. Gage and Fish (1924) also found that it was not digested. They suggested, as a possible explanation, the fact that ricinoleic acid contains more oxygen than do the ordinary fatty acids which are readily absorbed. Perhaps the same explanation may be applied to the soap as to the oil, but, since ordinary fat soaps are not absorbed, this appears to be a question of the soap group rather than of a specific soap.

The results with intraperitoneal injection of lard, butter, sodium ricinoleate and castor oil stained with Sudan IV indicated that these fats and soaps are not absorbed by the peritoneal surface. Castor oil was perfectly innocuous in the peritoneal cavity, in marked contrast to the castor oil soap.

Sodium ricinoleate is apparently not absorbed by the intestinal mucosa, hence any action it may exert should be limited to the lumen of the intestinal canal.

SUMMARY

1. The evidence indicates that oleic acid, butter, and lard are absorbed from the intestinal canal.
2. There is no evidence that soaps or castor oil are absorbed from the intestinal canal.
3. By the methods thus employed, butter, lard, lard soaps, castor oil, and sodium ricinoleate are not absorbed from the peritoneal cavity. Sodium ricinoleate appeared toxic in high concentration.
4. Any action of sodium ricinoleate administered orally would seem to be limited to the intestinal canal. Work is being continued in an effort to identify such action.
5. If the deaths of Rats 13 and 14 and the irritation in Rat 15 are an indication of absorption, we believe it is absorption of a material other than

the fatty acid radical stained with Sudan IV. No attempt to find other methods of absorption was made.

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SODIUM RICINOLEATE*†

II. A STUDY OF THE INTESTINAL FLORA DURING ORAL ADMINISTRATION

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SODIUM ricinoleate is used in gastrointestinal disorders which are believed to be due to a disturbance within the tract itself. To what extent these various disturbances are due to intestinal flora or to products of digestion is not known. This paper is a study of the effect of sodium ricinoleate, administered by mouth, on the intestinal flora.

Sodium ricinoleate was probably first used in bacteriologic work by Larson, Cantwell and Hartzell in 1919. They found a solution of sodium ricinoleate very effective in lowering the surface tension of media, by which the growth of bacteria was greatly influenced.

Albus and Holm (1926) reported that when the surface tension of the medium was lowered to forty dynes by means of sodium ricinoleate, the growth of *Lactobacillus bulgaricus* was inhibited, while *Lactobacillus acidophilus* grew well at thirty-six dynes.

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M. Frobisher (1926) found that surface tension is an important factor in bacterial growth. He used sodium oleate as a depressant but not sodium ricinoleate. W. R. Albus (1927), using sodium ricinoleate as a surface tension depressant, found that there was a decrease in the number of living cells in cultures of *E. coli*.

Pizarro (1927) found that inhibition of bacterial growth was not directly associated with surface tension. In many instances the greatest inhibition occurred at higher surface tensions, while broth at lowest surface tension gave the maximum development. This indicates that inhibition is due to the chemical nature of the depressant rather than lowering of surface tension. Day and Gibbs (1928) show that sodium ricinoleate is more toxic to bacteria than other soaps. They found that it is not the depression of the surface tension which affects the bacteria but some other action, possibly chemical. They reported that sodium ricinoleate exerted a more toxic action toward *L. acidophilus* than toward *L. bulgaricus*.

Kozlowski (1928) found that sodium ricinoleate in a dilution of 1:5,000 killed streptococci isolated from cases of erysipelas, measles, and scarlet fever in about seven hours, while control cultures survived for a week or more. Growth was inhibited by a dilution of 1:20,000 or less. Pneumococci were dissolved by a dilution of 1:5,000. *C. diphtheria* was inhibited by a dilution of 1:20,000 and killed by a dilution of 1:2,000 in seven hours. *S. paratyphosus*, *E. dysenteriae*, *E. coli*, and *Strep. fecalis* were very resistant to the bactericidal and inhibitory action of sodium ricinoleate. He concluded that specific cellular susceptibility of the bacteria and the concentration of the soap affects its bactericidal and inhibitory action.

Walker (1924) found the staphylococcus had a marked resistance to soaps in general. He found that the laurates had the greatest germicidal action of any soap. Pneumococci and streptococci were the most readily affected. He did not use sodium ricinoleate.

Spencer (1930) found that sodium ricinoleate, in appropriate amounts, completely cleared suspensions of certain species in normal salt solution, notably *B. tularensis*. He found that the density of suspensions of some other bacteria was increased by the addition of sodium ricinoleate and in still others the density would be increased at one dilution and decreased at another. He noted that many bacteria would grow in a 1 per cent solution of sodium ricinoleate. Upon observing the action of sodium ricinoleate upon *B. tularensis* with the dark-field illuminator, he found that the individual cells lose their refractility as a whole, but retain a few refractile granules for a time, which finally disappear, the resulting solution being noninfectious for guinea pigs.

The literature leaves undetermined the mode of action of sodium ricinoleate. There is no doubt that it has some effect upon bacteria but the mechanism is not clear. Apparently surface tension is not the only factor involved.

Selecting one of the most common members of the intestinal flora, *E. coli*, the percentage of sodium ricinoleate necessary to inhibit growth in cultures was studied. Plain broth was selected as one medium and a filtrate from stools of patients as another. The latter was chosen because conditions as

nearly as possible like those in the intestine were desired. Sodium ricinoleate was added to the media in varying amounts. The tubes were inoculated with a strain of *E. coli* isolated from a human source and incubated at 37° C. for twenty-four hours. Then a loopful was spread on a blood agar plate and incubated for twenty-four hours. Tubes which contained no sodium ricinoleate were used as controls. Tables I, II and III give the results:

TABLE I

SODIUM	TUBES			
Ricinoleate	1	2	3	4
10%	-	-	-	-
9%	-	-	-	-
8%	-	-	-	-
7%	-	-	-	-
6%	-	+	+	+
5%	+	+	+	+
0%	+	+	+	+
Plain broth media				

TABLE II

SODIUM	TUBES			
Ricinoleate	1	2	3	4
10%	-	-	-	+
9%	-	-	-	+
8%	-	-	-	+
7%	-	-	-	+
6%	-	-	-	+
5%	-	-	-	+
4%	+	-	+	+
3%	+	-	+	+
2%	+	-	+	+
1%	+	-	+	+
0%	+	-	+	+
Stool filtrate media				

TABLE III

SODIUM	TUBES			
Ricinoleate	1	2	3	4
8.3%	-	-	+	+
7.5%	-	-	+	+
6.6%	-	-	+	+
5.8%	-	-	+	+
5.0%	-	-	+	+
4.1%	-	-	+	+
0 %	+	+	+	+
1 c.c. stool filtrate plus 0.2 c.c. broth				

Table I demonstrates that at least a 7 per cent solution of sodium ricinoleate is required to completely inhibit the growth of *E. coli* in plain bouillon. In filtrate Tubes 2, Table II, there was no growth in any dilution nor in the controls. In two series of tubes in which nutrient broth was added to filtrate, the growth was inhibited by a 5 per cent solution of sodium ricinoleate while in a fourth series the organisms grew in every dilution. In Table III growth was inhibited in a 4.1 per cent solution in two series of tubes and in two others there was no inhibition in any dilution including the 8.3 per cent sodium ricinoleate. Thus, *E. coli* was quite resistant to the action of sodium ricinoleate, as far as growth is concerned.

The tubes in which there was growth in every dilution were found to have a white precipitate in the bottom resembling a calcium soap. These filtrates were found to contain considerable free calcium by quantitative test. The stools of ten patients who were receiving sodium ricinoleate by mouth were tested qualitatively for calcium, and it was found to be present in all, although in some in small amounts only. This suggests the possibility of sodium ricinoleate being sometimes precipitated as the insoluble calcium salt and rendered inactive when the drug is given by mouth and might indicate the use of much larger therapeutic doses.

In Part I of these papers it was found that the soap is apparently not absorbed from the intestinal tract. It would therefore appear that any action which the drug possesses must be local, and an attempt has been made to ascertain whether any local action can be detected by changes in the intestinal flora. In this, the effect of sodium ricinoleate on the general intestinal flora was studied by direct smear and by cultures. Cultures were made on blood agar plates, in brain broth and in cooked heart medium because each favors a different type of microorganism. No attempt was made to isolate and identify every variety because of the volume of work which would be entailed by such a procedure.

Sodium ricinoleate was administered orally in 5 or 10 gr. gelatin coated capsules. Thirty to 40 grains were given daily to each adult patient. The capsules were given thirty minutes before each meal and at bedtime, as the drug was better tolerated when the stomach was empty.

A specimen of stool was obtained from each patient before sodium ricinoleate was administered and as nearly as possible weekly thereafter. No stool more than five hours old was used. All glassware was autoclaved. Approximately 1 gm. of material from the center of the stool was emulsified with 75 c.c. of sterile isotonic sodium chloride solution. The emulsion was poured into an Erlenmeyer flask, shaken for one and one-half minutes, and centrifuged for thirty seconds at half speed, which was sufficient to throw coarse material down, but left bacteria in suspension. From this suspension, media were inoculated and slides made and stained by Gram's method. The P_H of each stool was determined by the colorimetric technic, using brom-thymol-blue as an indicator. For stools which were more acid, brom-cresol-purple was used. Sodium ricinoleate by mouth apparently did not influence the P_H of the stools. The patients were treated for eight weeks or longer. The direct smears from the stools were stained by Gram's method. Gram stains were made from the brain broth and heart media and from all varieties of colony on blood agar plates after forty-eight hours' incubation at 37° C. This was done prior to beginning the administration of sodium ricinoleate and each week thereafter.

Ten patients were studied in this manner and protocols kept. Table IV is a reproduction of one such protocol. It is considered unnecessary to include the others because, while there were minor variations, the general trends were the same.

TABLE IV
GRAM STAINED SMEARS. MRS. M.

DATE	P _H	STOOL	BLOOD AGAR	BRAIN BROTH	HEART MEDIUM
1932					
3-28	6.8	Coli	Coli Slender Gram+ rods	Coli predominant Few Gram+ rods	Coli Strep. Staph.
4-11	6.8	Coli Gram+ diplococci Large Gram+ rods	Poor growth Staph.	Coli Gram+ diplococci Few large Gram+ rods	Coli Many short coccoid Gram+ rods
4-18	6.6	Coli Gram+ cocci Acidophilus Welchii	Coli Gram+ spore former	Coli, Strep. Gram+ coccoid organisms in chains	Coli Gram+ cocci
4-25	6.4	Coli Gram+ diplococci Acidophilus	Coli	Coli Gram+ cocci Acidophilus	Coli Gram+ diplococci Welchii Gram+ club-shaped rod
5- 2	6.2	Coli Few Gram+ diplococci	Coli	Coli, Strep. Long Gram- rods	Coli Welchii Acidophilus
5-16	6.0	Coli Gram+ diplococci Acidophilus	Coli Small Gram+ rods	Coli Gram+ diplococci Acidophilus	Coli Welchii Gram+ diplococci
5-23	6.5	Coli, Strep. Acidophilus Gram+ diplococci	Coli	Coli Gram+ diplococci Small Gram+ rods in chains	Coli, Welchii Staph. Small Gram+ rods
6- 2	6.5	Coli Short Gram+ rods Large Gram- rods Gram+ cocci	Coli	Coli Gram+ diplococci Strep.	Coli, Welchii Gram+ diplococci Long Gram- rods
6-20	6.5	Coli Gram+ diplococci Welchii Small Gram+ rods Long Gram- rods	Coli	Coli Small Gram+ rods	Coli, Welchii Gram+ diplococci
Patient was given forty grains sodium ricinoleate daily for sixty-four days					

SUMMARY

The daily administration of 30 to 40 gr. of sodium ricinoleate by mouth for about eight weeks had no evident effect on the character of intestinal flora as far as was indicated by direct smear and the cultural characteristics observed. All types of bacteria grew as readily during the administration of sodium ricinoleate as before. The ratio of gram-negative to gram-positive organisms was not changed. In these patients every stool seemed to contain 90 per cent or more of gram-negative bacteria, and these were largely colon bacilli. The growth of all organisms in the media employed seemed just as prolific during the administration of sodium ricinoleate as when no medication was given. The P_H of the stools was not appreciably altered by sodium ricinoleate. Larger doses of sodium ricinoleate might be more effective, for some of the drug appears to be precipitated by the calcium in the intestinal

contents. This would be especially true in those cases where the P_H was quite low. That this could be done with impunity is indicated by the fact that doses which were comparatively several times larger were given to white rats for weeks with no ill effects. For instance, one gram was given daily to one rat through a stomach tube, and at no time did it affect its appetite or well-being.

CONCLUSIONS

1. *E. coli* was inhibited by 7 per cent sodium ricinoleate in broth culture.
2. Sodium ricinoleate, in the accepted clinical dosage, does not alter the intestinal flora as far as can be detected by direct smears and cultures in the three media employed.
3. Sodium ricinoleate in like dosage by mouth has no demonstrable effect on the P_H of the stool.
4. The gram-negative gram-positive ratio is apparently not changed by sodium ricinoleate.
5. The precipitation of sodium ricinoleate by calcium in the intestinal canal is worthy of study, as larger doses might be needed to replace that lost in this way.

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THE VARIATIONS OF THE UREA, TOTAL NONPROTEIN NITROGEN, AND CHLORIDE CONCENTRATION IN THE BLOOD FOLLOWING GLUCOSE INGESTION*

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HERRICK¹ and Katayama² observed a fall in the chloride content of whole blood following the ingestion of glucose in human subjects. An inverse relationship between the concentration of glucose and chlorides in the blood has also been noted in diabetes mellitus by Gram³ and McLean,⁴ also by Myers.⁵ In cases of Bright's disease, Gram⁶ has shown that the retention of nonprotein nitrogenous bodies is often accompanied by a reduction in the chloride content and conductivity of the blood. As in diabetes mellitus with high blood sugar, he assumes that the decrease in conductivity (i.e., salts) represents a regulatory process, which tends to stabilize the osmotic pressure of the blood. McLean⁶ has observed that there is a progressive diminution in the plasma chlorides in some patients with Bright's disease up to the time of death in uremic coma, and the presumption has been made by others that this decrease may be partly explained as an attempt to maintain an osmotic balance in the face of a rise of the nonprotein nitrogenous substances in the blood. Experiments reported by Ni⁷ show that the fall and rise of blood chlorides which occur in pancreatectomized or normal dogs after the injection of insulin are related to the sugar fluctuations, and he assumes such reciprocal changes to be an effort on the part of the body at osmotic and other compensations.

Such compensatory relationships are not uniformly observed during hyperglycemia and nitrogen retention and, as we shall attempt to show later, following the ingestion of glucose in normal and diabetic subjects. Mosenthal⁸ has recently demonstrated that the plasma cholesterol following glucose ingestion may either rise or fall markedly as the blood sugar increases, and consequently the assumption that a reciprocal variation of these two substances maintains the osmotic equilibrium of the blood does not suffice as an explanation for all changes. Experiments carried out in this laboratory on the variations of the plasma proteins during induced hyperglycemia (to be published later) show that the protein fractions of the blood may either rise or fall considerably under such circumstances. It is conceivable when reciprocal relationships between certain constituents in the blood were not observed, that other substances, the

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concentrations of which were not investigated, may have varied in such a way as to maintain a constant osmotic pressure of the blood.

MATERIAL AND METHODS

Sixty glucose tolerance tests were carried out in 54 unselected subjects. The variations of the sugar, urea nitrogen, total nonprotein nitrogen and chloride concentrations in the blood were followed at intervals, in most cases, of twenty minutes, forty minutes, one hour, two hours, and three hours following the ingestion of 100 gm. of glucose. The test was carried out in every instance in the morning, the subject having taken no food since the evening preceding the test. The volume output and the sugar content of the urine were also noted. The blood sugar was determined in every case and the patient's tolerance for ingested carbohydrate interpreted according to the standards proposed by Mosenthal.⁹ The variations of the urea nitrogen concentration in the blood were followed in 45 cases, the total nonprotein nitrogen in 13, the whole blood chlorides in 17 and the plasma chlorides in 6.

The methods employed were as follows: urea nitrogen, gasometric urease method of Van Slyke;¹⁰ total nonprotein nitrogen, Folin and Wu;¹¹ sugar, Folin and Wu;¹² whole blood and plasma chlorides, Short and Gellis.¹³

RESULTS

Urea Nitrogen.—Table I illustrates the variations of the urea nitrogen content of the blood in 45 subjects following glucose ingestion. Assuming deviations of ± 1.0 mg. per cent or more of urea nitrogen as significant, the following findings are demonstrated: *Progressive Fall*: (22 instances) Cases 5 to 8, 10, 13, 15, 17, 19, 22, 24, 25, 27, 30, 32 to 34, 37, 39, 41, 43, and 44. *Preliminary Rise and Later Fall*: (12 instances) Cases 2, 4, 11, 16, 20, 26, 28, 31, 35, 38, 40, and 42. *No Significant Change*: (6 instances) Cases 1, 9, 12, 21, 29, and 36. *Progressive Rise*: (4 instances) Cases 14, 18, 23, and 45. *Preliminary Fall and Later Rise*: (1 instance) Case 3.

Apparently, there is no constant reciprocal relationship between the urea and sugar content of the blood. Cases 5 to 8 and 10 show a persistent diminution in the urea nitrogen while the blood sugar has returned to the control level (normal sugar curves). Again, in Cases 31, 35, 38, 40, and 42 an initial rise and later fall in the urea nitrogen are associated with a persistently elevated blood sugar (high prolonged sugar curves).

A marked fall in the urea nitrogen content of the blood (3 mg. per cent or more) is observed in only one out of eleven subjects (9 per cent) with normal carbohydrate tolerance (Case 5). On the other hand, in the 25 subjects with definitely diminished sugar tolerance (high prolonged sugar curves), we record six (24 per cent) such instances (Cases 24, 27, 32, 37, 43, and 44). Two cases in each group show an increment of 3 mg. per cent or more in the urea nitrogen (Cases 3, 4 and 40, 45, respectively).

Our results demonstrate that following the ingestion of glucose the urea nitrogen content of the blood may rise or (and) fall or show no marked change. A diminution of 1 to 7 mg. per cent, however, occurs in about 50 per cent of the cases, and evidence is presented that an appreciable fall (3 mg. per cent or

TABLE I

THE EFFECT OF GLUCOSE INGESTION ON THE UREA NITROGEN CONTENT OF THE BLOOD

CASE	UREA NITROGEN (MG. PER 100 C.C.)							INTERPRETATION OF GLUCOSE TOLERANCE TEST	URINE VOLUME OUTPUT* C.C. PER MIN.	
	CON- TROL	20 MIN.	40 MIN.	1 HR.	2 HR.	3 HR.	MAXIMAL INCREASE ABOVE CONTROL			MAXIMAL DECREASE BELOW CONTROL
1	9.1	9.1		9.4	8.7	8.7	0.3	0.4	Normal	1.6
2	13.7	14.7		13.7	13.7		1.0	—	Normal	0.5
3	8.9	6.8	6.8	8.9	12.3		3.4	2.1	Normal	1.2
4	16.0	20.2		16.0	17.0		4.2	—	Normal	0.7
5	20.9	20.8	14.3	18.6	14.9		—	6.6	Normal	2.3
6	13.0	11.2	11.2	10.5	10.8	10.1	—	2.9	Normal	3.6
7	7.1	7.3	7.1	7.5		5.0	0.4	2.1	Normal	2.4
8	15.4	15.3	14.2	15.2	14.9	13.9	—	1.5	Normal	6.2
9	13.1	13.6	13.6	13.6	12.2		0.5	0.9	Normal	0.7
10	15.3	15.2	14.6	14.9	13.4		—	1.9	(Renal glycosuria)	
11	13.6	15.6	15.1	13.7	11.7		2.0	1.9	Normal	1.6
12	10.6			9.9	10.1		—	0.7	(Renal glycosuria)	
13	13.8	12.6	12.8	12.6	12.0		—	1.8	Low	1.4
14	15.4	17.1	16.3	17.3	17.0		1.9	—	Low	0.8
15	13.1	12.3		11.5	11.0		—	2.1	Low	2.7
16	11.6	12.9	14.1	13.8	10.8		2.5	0.8	High	0.8
17	12.7	12.2	11.6	11.9	10.4	10.9	—	2.3	High	0.9
18	9.0	10.1	9.6	9.0	10.1		1.1	—	(Renal glycosuria)	2.0
19	22.0	19.6		21.4	19.7		—	2.4	Prolonged	0.7
20	16.1	20.7	15.0	15.2			4.6	1.1	Prolonged	1.0
21	8.2			8.7	9.1		0.9	—	Prolonged	1.5
22	9.7	10.0	9.8	9.4	8.7	7.6	0.3	2.1	High prolonged	1.2
23	10.9	12.1	11.2	11.0	11.5		1.2	—	High prolonged	0.6
24	24.2	21.9	22.0	19.9	22.1		—	4.3	High prolonged	1.1
25	11.1	12.0	11.2	9.9	9.6	9.7	0.9	1.5	High prolonged	2.1
26	6.9	5.7	5.9	8.3	7.2	5.4	1.4	1.5	High prolonged	2.6
27	16.3	15.0	15.4	15.0	13.4		—	2.9	High prolonged	4.2
28	11.5	11.9	11.4	12.8	10.7	11.4	1.3	0.8	High prolonged	4.1
29		15.1		14.7	15.1		—	—	High prolonged	0.6
30	22.0	19.6		21.4	19.7		—	2.4	High prolonged	0.8
31	13.2	14.1	14.7	13.8			1.5	—	High prolonged	1.0
32	16.7	14.4	11.6	12.5	10.7	9.6	—	7.1	High prolonged	1.3
33	16.5	13.7	15.3		15.8		—	2.8	High prolonged	0.5
34	6.5	5.2					—	1.3	High prolonged	0.5
35	15.1	16.4	16.9	16.7	15.5		1.8	—	High prolonged	0.9
36	17.1	17.6	16.5	17.0	17.0		0.5	0.6	High prolonged	0.6
37	14.5			11.4	12.0		—	3.1	High prolonged	1.4
38	11.8	12.1		14.4	11.7		2.6	0.1	High prolonged	1.0
39	15.1	14.8	13.7	13.6	12.2		—	2.9	High prolonged	1.9
40	10.0			13.4	10.4		3.4	—	High prolonged	2.1
41	12.3	11.0		11.4	11.0		—	1.3	High prolonged	0.9
42	20.9	21.0	23.1	20.9	21.2		2.2	—	High prolonged	0.8
43	18.8	19.8		12.5	12.5		1.0	6.3	High prolonged	2.2
44	16.2	14.2	13.7	14.8	12.9		—	3.3	(Renal glycosuria)	1.2
45	11.9	13.4	12.4	11.9	16.2		4.3	—	(Renal glycosuria)	2.1
									(Renal glycosuria)	3.5

*Calculated from the total urine volume output following the glucose ingestion until the termination of the test.

more) occurs more often in subjects with diminished carbohydrate tolerance than in those exhibiting normal tolerance for sugar.

TABLE II
THE EFFECT OF GLUCOSE INGESTION ON THE NONPROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD

THE EFFECT OF GLUCOSE INGESTION ON THE NONPROTEIN NITROGENOUS CONSTITUENTS OF THE URINE																				
CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)						UREA NITROGEN (MG. PER 100 C.C.)				N.P.N.—UREA N (URIC ACID, CREATININE, AMINO ACID AND DEST N)						INTERPRETA- TION OF GLUCOSE TOLERANCE TEST	URINE VOLUME OUTPUT* C.C. PER MIN.		
	CON- TROL	20 MIN.	40 MIN.	1 HR.	2 HR.	3 HR.	MAXIMAL INCREASE ABOVE CONTROL	MAXIMAL DECREASE BELOW CONTROL	MAXIMAL INCREASE ABOVE CONTROL	MAXIMAL DECREASE BELOW CONTROL	CON- TROL	20 MIN.	40 MIN.	1 HR.	2 HR.	3 HR.			MAXIMAL INCREASE ABOVE CONTROL	MAXIMAL DECREASE BELOW CONTROL
1	29.5	27.6	26.9	26.9	29.3	26.9	—	2.6	0.3	0.4	20.4	18.5	17.5	20.6	18.2	2.9	0.2	2.9	Normal	1.6
6	31.6	33.0	31.9	31.1	29.1	29.0	1.4	2.6	—	2.9	18.6	21.8	20.6	18.3	18.9	0.3	3.2	0.3	Normal	3.6
7	30.0	29.3	33.2	33.2	29.1	24.5	3.2	5.5	0.4	2.1	22.9	22.0	26.1	19.5	19.5	3.4	3.2	3.4	Normal	2.4
8	35.6	34.2	32.7	33.0	31.6	30.7	—	4.9	—	1.5	20.2	18.9	18.5	16.7	16.8	—	—	—	Normal	6.2
12	31.4	31.4	35.1	27.3	27.3	—	3.7	4.1	—	0.7	20.8	20.1	21.5	17.2	17.2	4.4	4.4	3.6	Low	1.4
15	30.8	32.4	33.0	33.0	33.9	—	3.1	—	—	2.1	17.7	20.1	21.5	22.9	22.9	5.2	5.2	—	High	0.8
21	27.7	27.7	29.3	26.7	26.7	1.6	1.0	1.0	0.9	—	19.5	19.5	20.6	17.6	17.6	1.1	1.1	1.9	High	1.2
22	30.3	30.5	33.3	26.8	28.2	30.0	3.0	3.5	0.3	2.1	20.6	20.5	23.5	17.4	19.5	22.4	2.9	3.2	High	0.6
26	20.6	20.1	22.2	25.0	19.8	19.7	4.4	0.9	1.4	1.5	13.7	14.4	16.3	16.7	12.6	14.3	3.9	1.1	High	4.2
28	35.3	28.0	35.5	30.9	26.0	—	0.2	2.3	2.3	0.8	23.8	16.1	24.1	20.2	14.6	9.2	0.3	9.2	High	0.6
29	31.3	37.7	37.7	39.2	—	7.9	—	—	—	—	22.6	22.6	23.0	24.1	—	—	—	—	High	0.8
30	38.5	36.4	36.5	35.6	—	—	—	2.9	—	2.4	16.5	16.8	15.1	15.9	—	—	0.3	1.4	High	1.0
37	35.5	—	33.3	30.8	—	—	—	4.7	—	3.1	21.0	—	21.9	18.8	—	—	0.9	2.2	High	1.0

*Calculated from the total urine volume output following the glucose ingestion until the termination of the test.

Total Nonprotein Nitrogen.—Table II illustrates the variations of the total nonprotein nitrogen content of the blood and the sum total of the nonprotein nitrogenous substances excluding urea in thirteen subjects following glucose ingestion. The following findings are demonstrated:

1. The total nonprotein nitrogen may rise or (and) fall or remain unchanged.

2. The total nonprotein nitrogen shows a progressive fall in many instances and is paralleled by the variations of the urea nitrogen in Cases 6, 22, 26, 30, and 37. In these instances, the diminution in the total nonprotein nitrogen is due mainly to the decrease in the urea nitrogen fraction.

3. The sum total of uric acid, creatinine, amino acid, and rest nitrogen may either rise or (and) fall or show no marked change.

Whole Blood and Plasma Chlorides.—Table III shows the variations of the whole blood and plasma chlorides in seventeen and six cases respectively following the ingestion of glucose. In the six instances with plasma chloride studies, simultaneous determinations of the whole blood chlorides were made. The following findings are demonstrated:

1. A reciprocal relationship between the whole blood chlorides and sugar in Cases 46, 48, 50, 32, 52, 53, 55, and 56. In the first three cases, the fall and subsequent rise in the chlorides are associated with an increase of the blood sugar and a later return to the control level (normal and high sugar curves). In the latter five instances, the persistent diminution in the chloride accompanies a prolonged elevation of the blood sugar (high prolonged sugar curves).

2. Nine subjects show either no change or an augmentation of the whole blood chlorides.

3. The variations of the plasma chlorides parallel the whole blood chlorides in Cases 52, 55, and 56. In Cases 47, 48, and 49, the changes in the blood chlorides are due mainly to variations of the chloride content of the plasma.

DISCUSSION

In 1916, Hiller and Mosenthal¹⁴ showed that the ingestion of 100 gm. of glucose by normal individuals was not accompanied by hydremic plethora. We carried out hematocrit studies in a few instances, and we were able to satisfy ourselves that little if any blood dilution occurred. In fact, we observed a slight degree of blood concentration following glucose administration in some cases. The variations of the constituents investigated, therefore, cannot be explained by changes which develop in the water content of the blood caused by the ingestion of 100 gm. of glucose dissolved in approximately 250 c.c. of water.

If the variations of the urea and other nonprotein nitrogen fractions of the blood and of chloride, plasma proteins, cholesterol, etc., following glucose ingestion represent attempts on the part of the organism to maintain a normal osmotic pressure of the blood, then it becomes clear why one constituent (e.g., urea) may rise, fall, or remain unchanged while another substance or substances (e.g., chloride, cholesterol, etc.) may vary in such a way that the summation of their individual osmotic effects is constant. It is conceivable that the regulation is a property residing in all the body tissues and maintained by an interplay of the electrolytes and nonelectrolytes in tissue fluid and blood.

TABLE III
THE EFFECT OF GLUCOSE INGESTION ON THE CHLORIDE CONTENT OF WHOLE BLOOD AND OF PLASMA

THE EFFECT OF GLUCOSE INGESTION ON THE CHLORIDE CONTENT OF WHOLE BLOOD AND OF PLASMA																		
CASE	WHOLE BLOOD CHLORIDES (MG. PER 100 C.C.)						PLASMA CHLORIDES (MG. PER 100 C.C.)						INTERPRETATION OF GLUCOSE TOLERANCE TEST	URINE VOLUME OUTPUT* C.C. PER MIN.				
	CON- TROL	20 MIN.	40 MIN.	1 HR.	2 HR.	3 HR.	MAXIMAL INCREASE ABOVE CONTROL	MAXIMAL DECREASE BELOW CONTROL	CON- TROL	20 MIN.	40 MIN.	1 HR.			2 HR.	3 HR.	MAXIMAL INCREASE ABOVE CONTROL	MAXIMAL DECREASE BELOW CONTROL
46	464	406	473	453	461		9	58									Normal	7.9
12	419			483	419		64	—									Low	1.4
15	459	465		457	455		6	4									High	0.8
47	515	525	515	515	515		10	—	635	575	595	575	635	635	20	60	High	0.3
48	545	545	535	545	545		—	10	615	635	635	635	695	695	80	—	High	5.4
49	485	495	495	495	495	463	10	—	615	685	625	695				—	High	2.3
50	495	455	435	451	463		—	60	615								High	4.2
21	445			443	441		—	4									High prolonged	1.2
22	423	427	440	375	415	439	17	48									High prolonged	0.6
26	491	483	511	499	481	485	20	10									High prolonged	4.2
32	465	455	445	465	445	455	—	20									High prolonged	0.5
51	513	465		513	513		—	—	547	555		525	535	517	8	30	High prolonged	3.4
52	475	465		481	471	451	6	24									High prolonged	0.6
53	540	527	443	486	473	473	—	67									High prolonged	1.7
54	537	543	530	532	515		6	22	615	625	615	635	605	635	20	10	High prolonged	3.6
55	537	495	535	495	495		—	40	655	635	595	615	635		—	60	High prolonged	2.4
56	535																High prolonged	2.0

*Calculated from the total urine volume output following the glucose ingestion until the termination of the test.

No apparent relationship was observed between the degree of diuretic response to glucose and the variations of the several constituents in the blood. Urea, as a nonthreshold body, is capable of a "washing-out" process, but Mosenthal and one of us¹⁵ have shown that a marked degree of diuresis in human subjects does not influence materially the amount of urea cleared by the kidney in a unit of time. Obviously, the large amount of urea in tissues and blood is not affected appreciably by the increased rate of urine excretion. The interesting observation was made that diuresis following glucose administration occurred more often and was more marked in subjects exhibiting normal sugar tolerance than in those with diminished tolerance for carbohydrate. As a rule, mild and severe diabetics are dehydrated to a greater or lesser extent and for this reason it is probable that glucose ingestion in these cases is less apt to produce diuresis.

SUMMARY

The ingestion of 100 gm. of glucose by fasting subjects produced the following effects: (A) The urea nitrogen content of the blood may rise or (and) fall or remain unchanged. A gradual fall in the urea nitrogen (1 to 7 mg. per cent) was observed in 22 out of 45 cases. A definite decrease (3 mg. per cent or more) occurred more often in subjects with diminished carbohydrate tolerance than in those with normal tolerance. (B) The total nonprotein nitrogen of the blood may rise or (and) fall or show no marked change. In many cases, though not in all, the variations in the total nonprotein nitrogen were due mainly to changes in the urea nitrogen fraction. Irregular changes were noted in some instances in the sum total of the nonprotein nitrogenous substances in the blood other than urea. (C) There was a distinct reciprocal relationship between the whole blood and plasma chlorides and the glucose concentration in the blood in many instances, though this was not observed in all the cases studied.

There was no apparent relationship between the degree of diuretic response to glucose and the variations in the constituents investigated. Diuresis following glucose ingestion occurred more frequently and was more marked in subjects with normal carbohydrate tolerance than in those exhibiting a diminished tolerance for sugar.

The hypothesis is suggested that these fluctuations represent an effort by the body to maintain a constant osmotic pressure of the blood. The compensatory response of urea, total nonprotein nitrogen, and chloride to a rise of glucose in the blood is not a uniform reciprocal relation. The varied increase or decrease of these substances suggest that the total osmotic pressure is adjusted according to the physiologic reserve prevailing in the body at a given moment.

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THE PERENNIAL TREATMENT OF HAY FEVER*

A COMPARATIVE STUDY

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IN 1911, Noon¹ and Freeman² introduced the method of preseasonal desensitization of hay fever with specific pollen extracts. Their technic consisted of the subcutaneous inoculation into a sensitive patient of a number of increasingly potent pollen doses during the course of from ten to fifteen weeks preceding the date of expected pollination. While the merits of this treatment have since been generally recognized, the efficacy of their technic has been marred by the following objections: the inconvenience caused to the patient by the necessity of frequent visits to the office or clinic throughout the treatment period; the occasional need of a longer treatment period than allotted, in refractory cases; and finally, the rapid loss of the patient's acquired tolerance for pollens in the interim between treatment seasons.

In 1926 Zella W. Stewart³ suggested the "throughout the year treatment of hay fever," which, she claimed, met the above-mentioned objections effectively. By this method the patient is reinjected with an optimal dose of pollen extract at intervals of from two to four weeks throughout the year, thus maintaining his acquired immunity. In 1927 and again in 1932 Aaron Brown,⁴ working independently, described the "perennial" treatment of hay fever, for which he claimed similarly favorable results.

Additional observations on the perennial method of pollen therapy have since been made by Vander Veer, Cooke and Spain,⁵ Figley,⁶ Thommen,⁷ Vaughan,⁸ and Unger.⁹ In their final analysis these authors subscribed to all or most of the following advantages of the perennial over the preseasonal

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method of hay fever treatment: fewer office visits, better clinical end-results, greater convenience to the patient, and the possibility of permanent cures. They varied, however, on the incidence of constitutional reactions, which occurred more frequently by the perennial method in the series of Vander Veer, Cooke and Spain, and Thommen, as against a lesser incidence of similar reactions in the series of Figley, Vaughan, and Unger.

The magnitude of the problem of hay fever and the desire to elucidate further the merits of this innovation in its treatment prompted the undertaking of the study set forth in this communication.

Altogether 52 patients were treated by the perennial method during the year ending October, 1932. Of these, 34 patients had formerly received pre-seasonal treatment for one or more years and form the basis for comparing these two methods on the same group of patients in different years. At the same time 46 other patients who received preseasonal treatment in 1932 serve as additional material for comparing the perennial and preseasonal methods of treatment during the same season, though on different patients.

In Tables I, II, III, and IV, the results of our observations are tabulated under corresponding headings. It is evident that "complete relief" was about

TABLE I

A COMPARISON OF THE END-RESULTS IN 34 HAY FEVER PATIENTS TREATED PERENNIALLY IN 1932 AND PRESEASONALLY IN 1931

	1932 PERENNIAL	1931 PRESEASONAL
Complete relief	24 per cent	12 per cent
Marked relief	67 per cent	76 per cent
Slight relief	6 per cent	12 per cent
No relief	3 per cent	-----
Total per cent of good results	91 per cent	88 per cent

TABLE II

A COMPARISON OF THE AVERAGE NUMBER OF DOSES, AVERAGE AMOUNT OF POLLEN IN 1-100 DILUTION AND OF THE INCIDENCE OF CONSTITUTIONAL REACTIONS IN 34 HAY FEVER PATIENTS TREATED PERENNIALLY IN 1932 AND PRESEASONALLY IN 1931

	1932 PERENNIAL	1931 PRESEASONAL
Average number of doses	22	19
Average amount of pollen in 1-100 dilution	5.60 c.c.	2.04 c.c.
Constitutional reactions	0.06 per cent (1 reaction in 152 treatments)	0.07 per cent (1 reaction in 131 treatments)

TABLE III

A COMPARISON OF THE END-RESULTS OF HAY FEVER TREATMENT BETWEEN 52 PERENNIAL AND 45 PRESEASONAL CASES IN 1932

	PERENNIAL	PRESEASONAL
Complete relief	21 per cent	13 per cent
Marked relief	73 per cent	71 per cent
Slight relief	4 per cent	13 per cent
No relief	2 per cent	3 per cent
Total per cent of good results	94 per cent	84 per cent

TABLE IV

A COMPARISON OF THE AVERAGE NUMBER OF DOSES, AVERAGE AMOUNT OF POLLEN IN 1-100 DILUTION AND OF THE INCIDENCE OF CONSTITUTIONAL REACTIONS IN 52 PERENNIAL AND 45 PRESEASONAL CASES IN 1932

	PERENNIAL	PRESEASONAL
Average number of doses	24	21
Average amount of pollen in 1-100 dilution	5.89 c.c.	2.62 c.c.
Constitutional reactions	0.06 per cent (1 reaction in 156 treatments)	0.04 per cent (1 reaction in 236 treatments)

twice as frequent with the perennial method as compared with the preseasonal in both groups, while the instances of "slight relief" and "no relief" were more common in the preseasonal group. The amount of "marked relief," however, which the bulk of the patients experienced, was not appreciably different in the two groups. Altogether the group of 34 patients who were treated preseasonally in 1931 and perennially in 1932 obtained 88 and 91 per cent of good results in the respective years, while the other two groups, one of 45 preseasonal cases and the other of 52 perennial cases treated in 1932 experienced 84 and 94 per cent of good results respectively. For the slightly better total good results in the perennially treated cases, each patient received an average of three more doses and a total of about twice as much pollen as the preseasonally treated patients.

As seen from our tables, constitutional reactions occurred with slightly greater frequency in the 52 patients treated perennially in 1932 and in the 34 patients treated preseasonally in 1931. It is well to mention here that altogether we had 3 constitutional reactions which required adrenalin. Two of these occurred in perennially treated patients and one in a preseasonal patient. All other reactions were mild and needed no special medication.

TABLE V

ELEVEN CASES WHO OBTAINED BETTER RELIEF IN 1932 BY THE PERENNIAL METHOD

	1931 PRESEASONAL	1932 PERENNIAL
11 Cases	4 patients, slight relief 7 patients, marked relief	Marked relief Complete relief

FOUR CASES WHO OBTAINED WORSE RESULTS IN 1932 BY THE PERENNIAL METHOD

	1931 PRESEASONAL	1932 PERENNIAL
4 Cases	1 patient, complete relief 2 patients, complete relief 1 patient, marked relief	No relief Marked relief Slight relief

Of considerable interest is a group of 11 patients who obtained better relief in 1932 when treated by the perennial method than in 1931 when treated preseasonally. Against these we have 4 patients whose results in 1932 were inferior to those in 1931.

We were unable to standardize the time interval between treatments in the perennial group due to variation in changes in the individual patient's tolerance for pollen as determined by repeated serial skin tests and by the

extent of the local reactions from the previous dose. Most patients were treated at three-week intervals, some at four-week intervals, while a few patients could not maintain their tolerance any longer than two weeks. Two patients who had been refractory to treatment in the past received inoculations practically every week with excellent therapeutic results. In addition all patients were given weekly treatments throughout the pollinating season.

COMMENT

It is evident from this study that the perennial method of pollen therapy which is gaining considerable impetus in its clinical application is not infallible. While it offers a somewhat higher total of "good results" from treatment, it proved inadequate in about 12 per cent of our patients who had better relief when treated by the preseasonal method. Furthermore, in our series, the perennial method necessitated a larger number of visits per patient, and it showed no diminution in the incidence of constitutional reactions as compared with the preseasonal method. The one factor that seems to be decidedly in favor of the perennial method is the time element which permits of sufficient treatment in the refractory type of patient as evidenced by two of our cases who required inoculations at weekly intervals, throughout the year.

Of additional interest is the patient's personal reaction to this newer method of treatment. Those who could tolerate treatment at four-week intervals did not mind the occasional visits, while others who had to come in once every two weeks, resented the thought of the "endless visits to the doctor." They felt that eight or even seven months of freedom from treatment was a desirable period for relaxation.

Particularly unpleasant were those few instances when patients, though satisfied with the preseasonal method, acceded on our advice to the more trying perennial method, only to find the end-results less satisfactory. A case in point is the following: F. V. had complete relief in 1929, 1930, and 1931 by the preseasonal method of treatment, while in 1932, by the perennial method, his relief was only partial, although his maximum dose was the same each year. It is apparent that the frequency of administration of pollen doses may in certain instances influence the end-results in therapy.

As to the possibility of permanent cures by the perennial method, we cannot voice any opinion, since this study is based on the experience of one year's treatment. Should the permanent results ultimately prove to be better, one may disregard the several disadvantages incidental to the perennial method of treatment and advocate it with fewer reservations. For the present, however, we must accept this method only as "another method" applicable in selected cases, the most important indication for its use being refractoriness to treatment, where the time element is concerned.

Sight must not be lost of those instances pointed out by Rackemann,¹⁰ whose treatment by the preseasonal method may be reduced to six or even three doses a year, which is an impossibility by the perennial method in the best of hands.

Finally, a great many pollen sensitive patients are treated by the practitioner who may find the perennial method more complicated and less safe to the patient than the more simple preseasonal method of desensitization.

SUMMARY

A comparative study of the perennial and preseasonal methods of hay fever therapy indicate that:

1. The total good results from treatment were somewhat higher by the perennial method.
2. In about 12 per cent of the cases the results obtained by the perennial method were inferior to those produced by the preseasonal method of treatment.
3. The number of treatments per patient were greater by the perennial method.
4. Constitutional reactions occurred with slightly greater frequency in the perennially treated group.
5. The technic of treatment is far more complicated by the perennial method, while the convenience to the patient seems greater when treated by the preseasonal method.

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REFLEX EOSINOPHILIA*

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SEVERAL years ago one of us† produced emphysema with accompanying asthmatic syndrome in dogs by means of an intratracheal ball valve. Briefly, this condition was induced by the introduction of a ball valve into the trachea which would partially obstruct expiration without, in any way, affecting inspiration. Fig. 1 shows the valve in position in the trachea. This experiment led the authors to believe that a possible relationship exists between eosinophilia and mechanical expiratory dyspnea.

Previously, Freedman and Jackson¹ produced temporary expiratory obstruction in dogs for the purpose of studying the carbon dioxide values of the blood and of alveolar air. Their obstruction was brought about by employing a one-way valve attached to a tracheal T-tube. The results were obtained during experiments conducted under anesthesia.

Later, Pescatori² produced respiratory insufficiency by blocking the trachea with oil or tap water. Under these conditions he reported an eosinophilia which ran from 5 to 25 per cent. Unfortunately, it was impossible by this local edema method to maintain a constant tracheal pressure. Neither did this method permit regulation of the expiratory phase of respiration without affecting normal inspiration, because tracheal edema per se will alter both phases of respiration. Pescatori believes that the chronic stimulation of the tissues resulting from pathologic conditions, such as pneumothorax, pleurisy, etc., is of an acidotic nature and that this is the cause of the accompanying eosinophilia. To support this theory he poisoned rabbits rapidly with carbon dioxide (asphyxia) and obtained a high grade eosinophilia.

In our experiments blood studies were made before inserting the valve in the trachea of dogs to ascertain normal eosinophile values, and daily thereafter, during intubation. Further studies were carried during the acute asthma attacks and lastly at intervals following the spasms. The results of these studies are tabulated in Table I.

Our observation periods for dogs varied from twenty-four hours to seven days. During these periods aside from the expiratory dyspnea (asthma attack) which was purposely elicited (by exercise or the inhalation of tobacco smoke) for blood sampling, the general health of the animals was excellent.

In this table data are presented showing that, following the retardation of the expiratory phase of respiration, the eosinophile count increased on an

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†For complete detail consult "The Experimental Production in Dogs of Emphysema with Associated Asthmatic Syndrome by means of an Intra-Tracheal Ball Valve." J. Exper. Med. 30: 75, 1919.

average of 4 per cent, which was upon occasion increased by exercise or inhalation of smoke as high as 18 per cent. The type of respiration thus brought about produced in the animals every clinical point required for the diagnosis of asthma; and in addition, continued expiratory difficulty resulted in permanent emphysema.

In each case the total white cell count increased shortly after intubation, in two cases going as high as 15,000. This leucocytosis corresponds with that found in human beings during exercise. The eosinophile count increased to an average of 4 per cent on the first day, and to a maximum of 18 per cent in Dog 7 on the fourth day.

Each of these dogs exhibited eosinophilia after intubation, and developed a perfect pulmonary emphysema. Shortly after intubation fine whistling râles could be heard all over the chest, which were accentuated by exercise and

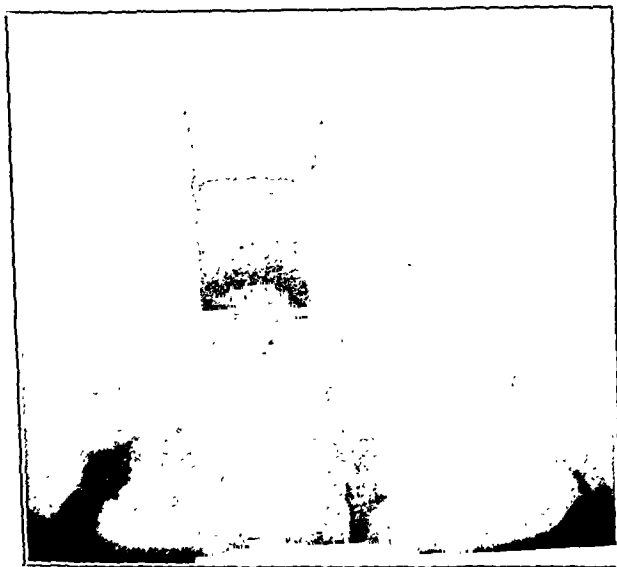


Fig. 1.—Dog 6, weight 12 kilos. Anteroposterior view of intratracheal ball valve in position.*

inhalation of irritants. This would indicate the added factor of bronchospasm, the total picture being identical with bronchial asthma. Of interest is the fall in eosinophile percentage in Experiment 6 following spontaneous extubation while physical signs of emphysema persisted. Reintubed this animal again showed an eosinophilia but of a higher grade. After exercise or inhalation of tobacco smoke, the eosinophilic count doubled; whereas, after extubation it remained at a normal level even though there were definite objective signs of emphysema.

The textbook³ statement that eosinophilia accompanies emphysema did not hold true in Dog 6 following valve removal, even though all the desired physical signs of emphysema such as extension of the areas of pulmonary resonance and hyperresonant percussion note over both lungs were present. In this experiment the typical wheezing prolongation of the respiratory murmur dis-

*Ma. 10, Time 3 sec. Kv. P. 45 Screens double. Distance 20" Film Eastman duplitzed.

TABLE I
TOTAL AND DIFFERENTIAL WHITE COUNTS ON INDICATED DAYS
AFTER INTUBATION

DOG	DATE OF INTUBATION	CONTROL COUNTS					1					2				
		TOTAL	P*	L	O	E	TOTAL	P	L	O	E	TOTAL	P	L	O	E
1	Oct. 28.....	8500	71	26	3	0	12500	76	20	0	4	11800	74	20	0.5	5.5
2	Nov. 3.....	6200	68	30	1.5	0.5	14000	80	15	1	3	12600	78	17	0	5.
3	Dec. 2.....	7000	68	29	3	0	13800	78	18	0	4					
4	Dec. 8.....	3800	65	34	1	0	9000	70	25	1	4	13100	75	20	0	5
5	Jan. 10.....	7600	70	28	1	1	14800	75	21	1	3	15000	76	19	0	5
6	April 15.....	7200	72	27	1	0	12600	68	29	0	3	12400	65	30	0	5
	May 16.....															
	reintubed.....	7400	68.5	31	0.5	0	13700	71	24	1	4	14200	70	22	0.8	7.2
7	Nov. 19.....	9680	67.1	24.3	8.6	0	16480	65	28	0.6	6.4	13200	69.6	16.	1.	13.4
												14000	71.0	15.	1.	13.0
												after	adren	alin	no	change

*P, Neutrophiles
L, Lymphocytes
E, Eosinophiles
O, Other cells

No significant changes were noted in total number of red cells, or in the hemoglobin per cent.

appeared with removal of the mechanical factor; and with the return of normal expiration the eosinophile count fell from 9 to 1 per cent. The degree of emphysema developed in this experiment is shown in Figs. 2 and 3, the first taken before intubation and the second six days later following five days of expiratory difficulty. The autopsy report* of this experiment which follows is also negative for eosinophiles, but confirms the clinical picture of emphysema.

PATHOLOGIC FINDINGS

"Specimen consists of two lungs. They are pale, soft, and crepitant. The margins are ballooned out, almost white in color and the alveoli are quite visible as minute cysts. Scattered throughout the upper lobe is a rather irregular mottling of the same process. The middle lobe is normal except along the margins and the same is true of the lower lobe.

"The second lung composed of four lobes shows a similar process, the margins are pale, noncollapsed. The upper lobes show a spotty pallor which becomes less and less noticeable in the lower lobe where only the marginal portion shows this distended condition.

"Diagnosis.—Emphysema: Bronchi are distended, very pale, contain no visible mucus or blood.

"Smears from bronchi show little mucus, desquamated epithelial cells, few mononuclear cells containing pigment. Smears for bacteria were negative, Wright's smear failed to show eosinophiles.

"The striking lesion in the parenchyma is the irregular dilatation of the alveoli, which is best seen toward the peripheral portion of lung tissue. Here, the alveolar walls are extremely thin and æmemic, and the alveolar spaces are three to four times as large as normal; bordering an area of alveolar dilatation are small patches of collapsed lung tissue.

"The bronchioles are distended.

"The walls of the vessels are normal except in a few branches of the pulmonary artery, which contain fibrin clumps that are undergoing organization.

"The glands along the bronchi and bronchioles as well as the mucosa are normal. The cilia stand out clearly, bathed by a very delicate layer of clear mucus.

"Occasional pigmented mononuclear cells are seen in the alveolar spaces and in the bronchial secretion.

"Differential count of white blood cells in large vessels shows normal count with no eosinophilia.

*For which we are indebted to the Department of Pathology.

TABLE I—CONT'D
TOTAL AND DIFFERENTIAL WHITE COUNTS ON INDICATED DAYS
AFTER INTUBATION

3					4					5					6				
TOTAL	P	L	O	E	TOTAL	P	L	O	E	TOTAL	P	L	O	E	TOTAL	P	L	O	E
11500	74	21	0	5.															
13500	75	19	0.5	4.5															
11500	65	30	0.5	4.5	10900	65	31	0	4	11000	65	29	1	1	8000	70	29	1	0
	60	31	0	9						tubed	coughed up				70	27	2	1	
13500	69	20	3	9															
14200	55	25.4	3.5	16.1	15400	56	22	4	18.0	12600	61	19	2.5	17.5	13000	60.5	21	1.5	17.0
					after	adrena-	lin	in	crease										

P, Neutrophils
L, Lymphocytes
E, Eosinophiles
O, Other cells

No significant changes were noted in total number of red cells, or in the hemoglobin per cent.

TABLE II

NO.	DIAGNOSIS	TOTAL	W.C.	E	NECROPSY	AGE
1	Emphysema, chronic bronchitis, chronic myocarditis	11,650		4.8	Necropsy	71
2	Emphysema, carcinoma prostate	12,200		0.5	Necropsy	58
3	Chronic bronchitis emphysema	9,480		4.6		52
4	Bronchial asthma emphysema	8,800		4.5		
		to 20,400		to 20.0		34
5	Chronic bronchitis emphysema, lobar pneumonia	10,400		6.0		
		to 36,000		to 4.2		60
6	Emphysema, chronic bronchitis	11,200		3.8		57
7	Emphysema, chronic bronchiectasis	16,200		3.8		51

TABLE III

BLOOD COUNTS IN HUMAN SUBJECTS

NAME	BLOOD COUNT BEFORE DYSPNEA				EXPIRATION PARTIALLY OBSTRUCTED	BLOOD COUNT DURING DYSPNEA				BLOOD COUNT 5 MINUTES FOLLOWING DYSPNEA			
	N.	E.	L.	O.		N.	E.	L.	O.	N.	E.	L.	O.
F. L.	62	0.5	32	5.5	1	71	2.0	24	3.0	66	0.5	29	4.5
M. H.	74	0	22	4	1	70	3.5	23	3.5	73	1	24	2.0
T. D. G.	66	0	30	4	2½	58.2	3.7	30	8.1	68	0	25	7
P. K.	68	0.5	25	6.5	1	69	4.0	23	4.0	70	1	26	3.0
K. G.	71	0	28.5	0.5	3½	66	4.5	26	8.5	70.5	1	28	0.5
M. W. B.	70	0	26	4	2½	72	6	20	2	71	0	25	4
E. S. R.	72.5	0	25	2.5	2	68	6.5	20.5	2.5	74	1	24.2	1.8
N. B.	71	0	26	3	3	66	6.5	24	2.5	74	0	23.5	2.5
P. B. H.	64	0	30	6	3	58.8	6.9	29.4	5.9	65	0	31	4
S. H.	66	1.5	30	2.5	1	71	8.0	19	2	70	2.0	26	2.0
L. S. N.	72	1	26	1	2	63	9.8	24.2	3	66.5	1.5	30	2
H. S.	74	1	24	1	3½	66.5	10.5	15.8	7.2	72	2.5	20	3.5
M. S.	72	1	25.5	2.5	2½	62	11	25	2	71	1.5	26	1.5
D. G.	73.6	0	22.7	3.7	2½	68	11	20	1	72	1.5	24	2.5
J. L. R.	70	1.0	21	8	3	58	14	22	6	67.5	3.5	20	9

"Examination of stroma surrounding bronchi and bronchioles shows no increase in cellular exudate and no infiltration of eosinophiles.

"Small fresh petechial hemorrhages are scattered throughout the lung parenchyma.

"Microscopic Diagnosis.—Parenchymatous emphysema, focal atelectasis; organizing thrombi in branches of pulmonary artery; petechial hemorrhages into alveolar spaces."

It is difficult to parallel this type of uncomplicated emphysema in human beings because such a condition does not call for hospitalization. Table II shows blood counts in selected cases. (All blood counts reported are averages of five or more taken at various times during the patient's illness.)



Fig. 2.—Dog 6. Anteroposterior view of chest before intubation, showing normal lung expansion.

In these cases of human emphysema all are complicated by pulmonary or bronchial pathology, with the exception of Case 2. This case was atrophic and of senile type, having no history of cough, and of interest since eosinophiles were practically absent in the blood smears.

The remaining cases all show a substantial eosinophilia. The high eosinophile count in the bronchial asthma case is explainable on the basis of bronchial spasm and expiratory dyspnea. In the remaining cases the emphysemas were complicated by chronic bronchitis coupled with a history of severe cough of long duration. During each cough there is a transitory overdistention of the lung, and this momentary obstruction to expiration is sufficient to cause both emphysema and eosinophilia and is similar to the eosinophilia and emphysema which we have produced experimentally.

In order to time the appearance of eosinophilia we studied the values produced by expiratory resistance upon medical students. Oral expiration was prolonged by employing a partially blocked flutter valve, the resistance offered varying from 20 to 30 mm. mercury pressure. Inspiration was through the nose. The results are tabulated in Table III.

The average time was two and one-fourth minutes, and the average degree of eosinophilia prior to and during dyspnea was 0.43 per cent and 7.19 per cent, respectively. In one-third of these cases the average eosinophilia was over 11 per cent. Hence, the eosinophilia occurring during dyspnea was approximately sixteen times greater than that prior to dyspnea.

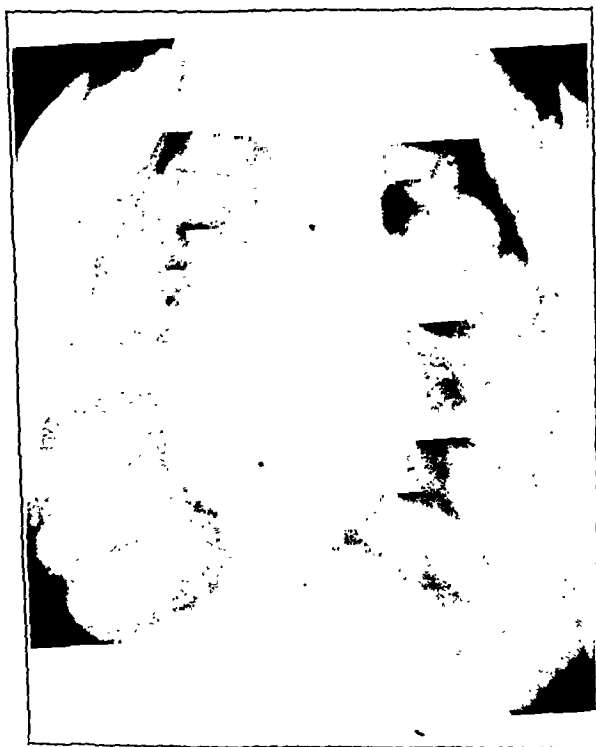


FIG. 3.—Dog 6, Anteroposterior view of chest six days after intubation showing extent of emphysema development.

We also believed it worth while to determine whether or not asthma, when temporarily relieved by adrenalin medication, was accompanied by changes in the eosinophilia count.

Table IV gives the results obtained in human cases, showing no eosinophile change under adrenalin. For completeness we have included negative results detailed in Table I, both as to spasm relief and eosinophilia change in dogs. One would expect lack of relief where mechanical conditions which from their nature and location would be the sole, specific and exciting cause of expiratory delay and alveolar distention.

TABLE IV
ADRENALIN ACTION IN HUMAN EOSINOPHILIA

PA- TIENT	HISTORY OF DIS- EASE	INITIAL COUNTS					ADREN- ALIN C.G.	COUNTS IN 15 MIN.					COUNTS IN 1 HOUR					COUNTS IN 24 HR.					COUNTS DURING RE- MISSIONS				
		TOTAL	P.	L.	O.	E.		TOTAL	P.	L.	O.	E.	TOTAL	P.	L.	O.	E.	TOTAL	P.	L.	O.	E.	TOTAL	P.	L.	O.	E.
1	Bronchial asth- ma. I. C. aged 34. Duration 8 yr. Attacks se- vere. Sensitive to wheat.	11,800	59.5	20	2.5	18	0.4 (no re- lief)	18,000	60	21	2	17	19,000	61	18.5	2	18.5										
							1.0 (re- lieved)	18,280	62	20	1.5	16.5	14,200	63	20	0	15.0	11,200	58	36	0	6	9,660	64	31	2	3
2	Bronchial asth- ma. V. M. aged 46. Onset after influenza 12 yr. ago. At- tacks moder- ate.	12,600	68	22	1.0	9	0.8 (re- lieved)	13,000	66.5	23	1.5	9	11,000	68	22	1.5	8.5	8,800	65	30	0	5	7,860	66	28	4	2
3	Chr. eczema. J. A. C., aged 18. Duration 6 yr. Skin tests reveal hy- persensitivity to egg yolks	8,200	65	30	0	5	0.8	8,200	64.5	30.5	0	5.5	8,060	65	29	1	5	8,400	66	30	0	4					

Camp⁴ has reported that the eosinophiles are markedly increased by adrenalin dosage. Our investigation of this phase is negative both in human beings and dogs, and we are unable to account for his positive findings.

That the other human cases show an eosinophilia is explainable on the basis of accompanying respiratory difficulty.

The possibility of adrenalin raising the eosinophilic index had proved negative in our tests as shown in Table I, and also in Table IV.

In this short series of human cases of allergic disease accompanied by eosinophilia there was no fall in eosinophilic count many hours after adrenalin, even though the dosage was adequate to give symptomatic relief. This continued eosinophilia rules out the possibility of delayed adrenalin action.

DISCUSSION

Prior to the experiments of Pescatori,² Freedman and Jackson¹ showed that the CO_2 content of the blood and alveolar air is raised when expiration is partly obstructed, but these authors did not investigate the blood picture. One would expect such an increase in CO_2 tension when the retarded expiration was reinforced by an anesthesia.

The theory advanced by Pescatori, that the increase in eosinophiles is brought about by the above conditions, is based upon poisoning rabbits rapidly with CO_2 (asphyxia). By this method he obtained a high degree of eosinophilia, i.e., 35 per cent.

In the production of asphyxia there is a condition which closely parallels our mechanical retardation of expiration, because certainly the second stage of asphyxia is characterized by expiratory convulsions.

The cause of this sudden and apparently selective action accompanying bronchospasm, we believe, is due to the overdilatation of the alveoli which, in turn, initiate reflex peripheral stimuli which brings about a release of eosinophiles from blood-forming organs. Garrey and Butler⁵ believe a similar mechanism is accountable for the temporary leucocytosis resulting from sudden distention of the stomach.

The degree of individual response varied, as shown in Table III, but the striking phenomenon is that in all cases sudden definite eosinophilia developed. The rapidity of this eosinophilic release is indicative of reflex action upon blood-forming organs and blood reservoirs. The source of these eosinophiles is probably the reservoirs because of the rapidity of response and the maturity of the cells (multi lobulated nuclei).

The leucocytic increase in our experiments is definite. If there were nothing specific in the release of eosinophiles their increase should be proportional to the leucocytosis. But the percentage increase in leucocytosis is relatively small compared to the percentage increase of eosinophiles, which again suggests that a release of eosinophiles is probably due to some specific entity.

Additional evidence that CO_2 (chemical stimulation) is not the factor is to be found in the uncomplicated case of emphysema in Table IV: also in Animal 6, Table I, following extubation. In both of these cases the eosinophilic index is not raised although it has been shown by Dautraban⁶ and others that in emphysema there is an increase in CO_2 tension.

Recently Martin,⁷ in his study of the effects of exercise upon leucocytosis, investigated also the reactions of the white cells under adrenalin. His results show that both exercise and adrenalin increase the total white blood cell count, principally the lymphocytes and the polymorphonuclear cells. Investigation of the effects of exercise upon the eosinophilic index was negative, although our total counts confirmed Martin's conclusions. Analysis of Martin's tables show that under adrenalin medication the greatest individual increase of eosinophiles was 1 per cent. In this case the initial count was 2 per cent before adrenalin and 3 per cent after. Other cases in his series show decreases under adrenalin, while the remainder show increases varying from 0.05 to 0.75 of 1 per cent. Certainly the minor increases reported by Martin could not be considered clinical increases, nor do we feel that his reported decreases up to 1½ per cent under adrenalin are of significance. One would expect just such slight variations in individuals.

As a result of our experiments a physiologic explanation is to presume that there is a temporary eosinophilia during the prolonged and forceful expiratory act, as for example, in whooping cough, blowing of wind instruments and glass blowing. That the data relative to eosinophilia in these conditions are lacking is explained by the fact that counts have not been carried out during the period of expiratory exertion.

It is an established custom for clinicians to associate eosinophilia as a definite blood finding of allergic phenomenon. We contend that the "allergic doctrine" confuses cause and effect, because in so-called allergic conditions there is frequently bronchospasm coupled with alveolar overdistention which produces eosinophilia. Eosinophilia, per se, does not suggest certain protein avoidance nor does it demand sensitizing tests.

CONCLUSIONS

1. Eosinophilia is produced by expiratory delay when accompanied by overdistention of the alveoli.
2. Evidence is offered which suggests that this response is specific and of a reflex nature.
3. Adrenalin does not affect the eosinophilic index.
4. The evidence available does not warrant the consideration of acidosis as a causative factor in eosinophilia.
5. Physiologic leucocytosis is not accompanied by eosinophilic increase.
6. Eosinophilia, per se, is not pathognomonic of allergic diseases.

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FURTHER STUDIES ON THE CULTIVATION OF *ENDAMEBA HISTOLYTICA* AND A COMPLEMENT FIXATION TEST FOR AMEBIASIS*

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I. CULTIVATION OF *ENDAMEBA HISTOLYTICA*

THE general belief that *Endameba histolytica* does not ordinarily ingest intestinal bacteria was contradicted by Boeck and Drbohlav¹ on the basis of their cultural experiments. They showed that the amebas were able to ingest bacteria, and consequently, they believed that the organisms might thrive without access to tissue elements. The work of Dobell and Laidlaw² as well as Cleveland and Saunders³ seemed to strengthen this contention. Dobell claimed that certain particular types of intestinal bacteria were especially favorable sources of food supply for the amebas. By the addition of such organisms, he succeeded in maintaining a cultural cycle of the amebas in vitro. Cleveland and Saunders cultivated the amebas from apparently bacteria-free amebic abscesses in the medium of Cleveland and Collier⁴ and found that the amebas were able to multiply very rapidly when *Escherichia communior*, *Vibrio comma*, and *Neisseria catarrhalis* were present.

Though Kofoed and Wagener⁵ believed that either coagulated egg or blood was required as a source of food for the amebas, Yorke and Adams⁶ considered moisture and a suitable temperature (37° C.) to be the only essential factors in inducing excystation of the organism in vitro. They reported that excystation occurred when cysts were placed in either Locke-serum, broth, or physiologic saline and incubated at 37° C. for a few hours, but a total disintegration of the organisms occurred in these mediums after several hours' incubation. In view of these findings it occurred to the author that the addition of suitable substances to one of these media might result in the survival of amebas beyond the stage of excystation.

The purpose of this study is to describe a method of cultivation of amebas, worked out with these experiments as a background. The details of the method have been somewhat modified since the original report on the subject.⁷

The method developed consists of the inoculation of washed cysts into a medium tentatively called S. C. medium composed of nutrient broth and a mixture of starch and charcoal with and without Dorsett's egg medium.⁸

Constituents of the Medium.—1. *Nutrient Broth* consisted of peptone 10 gm., meat extract 3 gm., and NaCl 5 gm. in one liter of distilled water adjusted to P_H 7.0 and autoclaved at 15 pounds' pressure for thirty minutes. The P_H of the medium within the range of 6.8 and 7.4 also answers the purpose, though the optimum is found to be 7.0.

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I wish to express my gratitude to Doctor J. Bronfenbrenner for his valuable advice and criticism during the course of this study.

2. *The S. C. Mixture*.—This is a thoroughly triturated mixture of rice starch and animal charcoal in the proportion of 2:1 by volume. A small amount of the mixture is loosely placed in a small vial and sterilized by dry heat at 180° C. for forty-five minutes.

Rice starch provides the amebas with a definite source of assimilable carbohydrate and inhibits the growth of *Blastocystis* frequently met with in feces. The addition of rice starch by Dobell and Laidlaw to the original medium of Boeck and Drbolav has been generally considered as a decided improvement, as extremely small grains of rice starch are readily ingested by the amebas.

Animal charcoal is used to adsorb ammonia and hydrogen sulphide thus reducing their deleterious effects upon the amebas, and inducing a partial anaerobiasis in the medium by the adsorption of oxygen. The relative value of charcoals of various origins have been studied. Merek's purified animal charcoal and Norit were found to be superior to others for this work. The calcium phosphate contained in these charcoals acts as a buffer, and also helps to stimulate the metabolic activities of the amebas. It was found that the addition of excess calcium phosphate to the medium did not materially increase the growth of the amebas, but on the contrary stimulated a marked proliferation of the accompanying bacteria, so that the amebas were crowded out.

Preparation of Washed Cysts.—This is carried out best according to the method of Hegner.⁹ Formed stool is thoroughly mixed by means of a sterile glass rod or spatula in order to obtain a uniform distribution of cysts. Liquid stool should first be sedimented, and the supernatant fluid carefully decanted. A portion of stool or sediment thus obtained is emulsified with a large quantity of water. This is filtered through four layers of gauze into a tall cylinder and allowed to stand for a few hours during which time cysts settle to the bottom. The process is repeated at least three times, until the sediment is found to be composed of the cysts and a small amount of debris. The sediment thus prepared, as a rule, contains fewer bacteria than are present in original stool. There is an especially noticeable reduction of the starch-splitting types of organisms, as is indicated by the presence of numerous unchanged starch granules in the medium after incubation.

When sufficient time is not available for the preparation of washed cysts, a portion of the sediment obtained from the preliminary filtration is transferred to a 15 c.c. centrifuge tube. By an addition of sufficient distilled water, this is washed by means of centrifugalization at 1,500 r. per minute for three minutes. This process should be repeated at least three or four times. Cysts, thus collected, were found to grow in the culture medium almost as well as those obtained by the slow sedimentation.

The author¹⁰ recently found that washed cysts remained viable as long as nine days when left at room temperature (22° C.) and thirty-five days in a refrigerator. Furthermore, irrespective of the stages of development of these cysts, they were found to grow to maturity in vitro under favorable environmental conditions. Hegner, Johnson and Stabler¹¹ observed that immature cysts were able to excyst in the small intestines of monkeys within three hours after being introduced into the stomach. A successful cultivation in vitro,

therefore, appears to depend on the number and viability of cysts inoculated, but not upon the stage of development of these cysts as previously considered.

Primary Cultures.—At the outset of this investigation, it was thought that an introduction of a small bit of an infected stool into the medium was sufficient to induce a good growth of the amebas. Subsequent studies, however, indicated that washed cysts were superior for the cultivation irrespective of the number of cysts found in the original stool. Apparently, the growth of the amebas was prevented by multitudes of bacteria present in the original stool.

A small amount of washed cysts (0.1 c.c. or less dependent upon the number of cysts present) is introduced into 8 c.c. of the broth previously warmed to body temperature, and two 4 mm. loopsful of the S. C. mixture are added. The tube is now incubated at 37° C. After an incubation period of from twenty-four to forty-eight hours, the sediment is at first gently seraped off from the bottom of the tube by means of a pipette with a wide terminal opening, and then about 0.1 c.c. or less is withdrawn carrying with it minute particles of charcoal. The amebas are usually found adhering to the particles of charcoal at the bottom of the tube. The organisms, if few are present, therefore, may readily escape detection in a material without charcoal. The contents of a pipette are spread on a warm clean slide, covered with a cover glass, and examined by means of a 10 x eye piece and 16 mm. dry objective, preferably either on a warm stage or in a warm chamber. Some of the amebas, at first seemingly quiescent, soon begin to project their pseudopodia explosively. Within a few minutes some of them are found crossing the microscopic field with rapidity and in a definite direction in an apparent search for food. As a rule, within the cytoplasm of the motile amebas there are observed fewer starch granules than within the quiescent ones. The amebas, if kept warm and moist, exhibit markedly progressive locomotion, as observation continues. The addition of a small drop of 0.1 per cent neutral red helps to differentiate the ectoplasm from the endoplasm without affecting the motility of the amebas, and facilitates the examination greatly, as it gives the endoplasm a pinkish refractive tinge as contrasted with other objects in the microscopic field. Care should be taken to guard against air currents and desiccation of the specimen, as this may interfere with the motility of the amebas, thus inducing them to round up and die.

By the use of this medium, the amebas may often be seen at various stages of development; excystation, metacystic development and encystation as well as division frequently occur simultaneously. The extent of growth of the amebas in this medium is very striking. Thus, by the introduction of 80 cysts, the number of the trophozoites was calculated to be 21,200 after forty-eight hours' incubation in one instance, while, in other instances, 40 cysts gave rise to 13,900 and 72 cysts to 12,200 trophozoites.

While the medium seems to be very favorable one for *E. histolytica*, it does not seem to support the growth of other protozoa. Repeated attempts to grow *E. coli*, *E. nana*, and *I. williamsi* in this medium were without results. The same also holds true to a certain extent with the flagellates such as *Trichomonas hominis* and *Chilomastix mesnili*; these organisms grow quite readily in the absence of the S. C. mixture from this medium. Though no plausible explanation can be given at this time, it is suggested that P_H of the medium and the

nature of the bacterial contents, as well as the oxygen tension may not be suitable for these organisms.

Subcultures.—Subcultures may be made by inoculating a small amount of the sediment from the primary culture to a fresh tube of the original culture medium previously warmed to body temperature. Transfers should be made at least every forty-eight hours in order to induce a good growth.

It has also been found that when about 5 c.c. of the S. C. medium is superimposed on a slant of Dorsett's medium, an efficient medium for subculturing and primary cultures* is obtained. With careful manipulation, this medium serves to maintain luxurious growth of the amebas through many generations when transfers are made at twenty-four-hour intervals. The amount of the inoculum should not be greater than 0.05 c.c., as the excess often leads to the overgrowth of the accompanying bacteria which interferes with the optimum cultural environment for the amebas.

Maintenance of Cultures.—When washed cysts are first exposed to the action of a mixture of equal volume of gentian violet and acriflavin (1:100 dilution each) at 37° C. for one hour, and then inoculated into the S. C. medium on Dorsett's medium slants, the amebas usually maintain growth for as long as eight days without further transfer. In these circumstances the lag period before detectable evidence of growth is prolonged, but the development thereafter is remarkably luxuriant. The long lag period may be accounted for by the amebastatic activities of these dyes, such as had been observed by Churchman¹² in the study of bacteriostasis. The detailed study of this problem will be reported at a later date.

Diagnostic Value of the Culture Method.—The superiority of the cultural method over the direct microscopic examination and concentration methods for detection of amebas in stools was clearly shown by the fact that, of 346 stools examined at random for intestinal parasites, 5, or 1.44 per cent were positive for *E. histolytica* by the direct microscopic examination of the stools, 7, or 2.02 per cent, by the concentration method of Rivas,¹³ and 9, or 2.60 per cent, by the use of this medium. In each instance, the final verification of the organism was made by permanent preparations which were stained by the method recently described by the author.¹⁴ The cultural method, if properly employed, therefore, is of considerable value in the routine diagnosis for the presence of *E. histolytica*. Furthermore, the fact that other endamebas failed to grow in this medium adds to its value for diagnostic use.

II. COMPLEMENT FIXATION TEST FOR DIAGNOSIS OF AMEBIASIS

Craig¹⁵ has reported positive complement fixation tests in 92 per cent of a group of 84 cases of amebic infection, all of which showed the amebas in the stools. Recently, Keifer,¹⁶ aided by Craig, reported strongly positive complement fixation reactions with the same antigen (prepared from amebic culture in Boeck and Drbolav medium) in 15 out of 19 cases of chronic ulcerative colitis, but neither feces nor materials obtained by means of the proctoscope revealed the presence of the amebas in these cases. The question naturally arises as to the

*See additional note in abstract, Further Studies on the Cultivation of *Endameba histolytica*, J. Parasit. 20: 143, 1933.

significance of the positive reactions in these instances. They might conceivably be due to the presence of amebic lesions outside of the intestine. It is possible, though unlikely, that the positive reactions were caused by existence of complement fixing antibodies in the patient's serum for the bacteria in the antigen preparation used. Keifer suggested the possibility that the pyogenic infection of the colon in these cases might be superimposed upon a preexisting amebic ulceration and cited in support of this the disappearance of symptom in some of his cases following antiamebic therapy. There is, however, no direct evidence that infection with the amebas was the causative factor in any instance. The beneficial effect of emetine therapy by no means constituted proof of amebic infection.

The purpose of the present study is to further investigate the value of complement fixation tests in diagnosis using as an antigen the culture in the medium devised by the author.

Reagents.—One of the obstacles in perfecting the complement fixation procedure is the difficulty in separating the amebas completely from the accompanying bacteria in vitro so as to prepare a specific antigen. The selection of material suitable for preparation of the antigen requires utmost consideration, particularly with reference to (1) the number of bacteria in the original stool in proportion to that of the amebas, and (2) the choice of a culture method which will further reduce the number of bacteria and will stimulate the growth of the amebas.

Antigen: For the preparation of the antigen, Craig¹⁷ utilized the Boeck-Drbolav medium¹ containing 2 or 3 amebas per microscopic field, while Menendez¹⁸ used the material containing 8 or 10 per high power microscopic field. Hage¹⁹ in his attempt to confirm Izar's²⁰ work with the complement fixation reaction attributed his failure to a lack of sufficient number of amebas to be extracted for the aqueous antigen. Because of the small number of amebas present Craig used in his work an undiluted antigen. The number of the amebas in the antigen in my study was considerably greater than in those of Craig and Menendez; the average number per high power microscopic field being 54. Repeated titrations of the antigen indicated that dilutions lower than 1:100 showed anticomplementary property. One-fourth of the anticomplementary unit of this 1:100 dilution was employed in the test.

Owing to the presence of the amebas only on or below the level of charcoal in the S. C. medium, no preliminary centrifugalization was deemed necessary to obtain the material to be extracted. The supernatant fluid above the charcoal was carefully pipetted off. The sediments of at least 24 cultures including the charcoal and starch were collected and placed in a conical centrifuge tube with an addition of warm normal saline solution. This was repeatedly centrifuged at slow speed, until the sediment contained a comparatively small number of the accompanying bacteria. Usually, seven or eight centrifugalizations were necessary to accomplish this end. By following the method of Craig, to one volume of the sediment was added ten volumes of absolute alcohol. The mixture in a tube, hermetically sealed, was left in an incubator at 37° C. for fifteen days, having been shaken vigorously at least three times a day during this

period. At the end of the period, the mixture was filtered through a fine filter paper, and the filtrate kept in a refrigerator until used.

In addition to the above, a second antigen was prepared by evaporating 10 c.c. of the above extract in an incubator at 37° C. until 1 c.c. mark was reached, and the evaporated portions replaced with normal saline solution.

As controls, extracts were also prepared in a similar way from the accompanying bacteria in the culture medium.

These antigenic preparations were titrated about every three weeks in order to check their potency.

Other Reagents: The hemolytic system used consisted of a 10 per cent sheep cell suspension, guinea pig complement (40 per cent) and rabbit antsheep amboceptor.

Test Proper: In this study, the ice box method of complement fixation was employed. To 0.1 c.c. of unknown serum, previously inactivated at 56° C. for half an hour was added one-fourth of anticomplementary unit of the antigen, and 2 units of complement. The tubes were left in a refrigerator for eighteen hours. One-tenth cubic centimeter of 10 per cent red cell suspension and 2 units of amboceptor were then added to each tube, and the incubation carried out in the water-bath at 37° C. for half an hour. Controls were run with known positive sera from cases of proved amebic infection and with normal sera, and the usual reagent controls. In the earlier part of the work the patient's sera were tested in various dilutions. The final readings were made after allowing red cells to settle sufficiently to the bottom of the tubes. For this purpose, the tubes are usually left in a refrigerator for two hours after the removal from the warm water-bath.

RESULTS AND DISCUSSION

TABLE I

CORRELATION BETWEEN RESULTS OF COMPLEMENT FIXATION TESTS AND ORDINARY DIAGNOSTIC METHODS*

CLINICAL DATA	TOTAL CASES	COMPLEMENT FIXATION POSITIVE. ORDINARY DIAGNOSTIC METHOD POSITIVE	COMPLEMENT FIXATION NEGATIVE. ORDINARY DIAGNOSTIC METHOD NEGATIVE	COMPLEMENT FIXATION POSITIVE. ORDINARY DIAGNOSTIC METHOD NEGATIVE	COMPLEMENT FIXATION NEGATIVE. ORDINARY DIAGNOSTIC METHOD POSITIVE
No evidence of amebiasis	130	—	130	—	—
Amebic dysentery	6	5	—	—	1
Carrier of <i>E. histolytica</i>	8	8	—	—	—
Suspected amebiasis	4	—	—	4	—
Ulcerative colitis	5	—	5	—	—
Totals	153	13	135	4	1

*Includes microscopic concentration and cultural examinations of stool and of material obtained by proctoscope (in some cases).

The comparison of examinations of stools and materials obtained by proctoscope with the findings in complement fixation tests with *E. histolytica* are shown in Table I. Both the feces and sera were found to be negative in 135 instances. In 13 cases of diagnosed or suspected infection the results were

positive both in feces and serum, while 4 gave a positive serum with a negative stool and one a negative serum with positive stool finding.

Judging from these observations, a negative reaction would appear to be quite significant if the tests were employed in the routine diagnosis, and would be of value in eliminating the possibility of amebiasis in a given individual. However, if repeated examinations were carried out, the number of positive stool findings might have been higher than that reported in this study. The author⁹ recently observed the presence of definite encystment cycle in a carrier of *E. histolytica*, as he had recognized in those of *Giardia lamblia*.²¹ Owing to considerable fluctuations in the number of cysts discharged from day to day, it was suggested that the examination of stools be made on alternate days for a longer period, rather than six consecutive days as advocated by Dobell.²² The necessity of frequent examination cannot be overemphasized, as is illustrated in the following instance:

In a patient giving the history of protracted diarrhea accompanied by the profuse discharge of blood and mucus, the repeated complement fixation tests were consistently positive. The repeated examinations of stools and of materials obtained by means of proctoscope failed to show any amebas until finally on the fourth examination (a warm specimen), motile amebas were detected. Among eight individuals free from clinical symptoms but known to be carriers of *E. histolytica* and giving positive complement fixation tests, the cysts were detected in the stools only after repeated examinations in some instances.

Of the 6 patients with amebic dysentery 5 exhibited strongly positive complement fixation reactions and the initial stool examinations revealed presence of *E. histolytica*. These patients showed ulcerative areas about the rectum and sigmoid which teemed with actively motile amebae ingesting many red blood cells. The remaining case of amebiasis (with positive stool) never showed any capacity to fix complement in repeated trials.

With respect to the 4 cases with negative stools but positive complement fixation reaction, the following possibilities may be considered: (1) the amebas might have become localized in the tissues only, while the intestinal lumen was devoid of their presence (such may occur in case of metastasis to other parts of body), (2) there may have been an insufficient number of stool examinations to reveal the amebas, or (3) the amebic lesions may have been too high in the intestine to permit detection by the proctoscope. Such lesions are frequent, judging from the works of Hutchinson²³ and Callender.²⁴ Clark²⁵ reported in his study of 186 postmortem examinations in the Panama Canal Zone that the ulcers were most frequently found in the cecum, the portion where the greatest stasis existed. In such, no ulcerations were detectable by the proctoscopic examinations. Low²⁶ claimed that the practical value of sigmoidoscopic diagnosis is very much limited, as autopsy often revealed dysenteric lesions in the cecum and other portions of the large intestine with no rectal involvement.

It is interesting to note that in the five cases diagnosed clinically as chronic ulcerative colitis both complement fixation tests and repeated microscopic examinations revealed no evidence of amebic infection.

In the majority of the cases showing positive complement fixation, there were concomitant intestinal protozoan infections with organisms such as *Giardia lamblia*, *Chilomastix mesnili*, *Endameba coli*, *Endolimax nana* and *Iodameba williamsi*. These, however, did not influence the serologic findings. Among those individuals giving negative tests, there were several who harbored intestinal protozoa other than *E. histolytica*.

The results of the complement fixation reactions carried out using bacterial extracts as antigen consistently gave negative reactions irrespective of the outcome of the reaction with the amebic antigen. This confirms the findings of Craig as well as Sherwood and Heathman.²⁷ However, the bacterial extracts prepared in this study cannot be considered quite adequate, since the bacterial flora is variable according to individuals, and from time to time in the same individuals. It is still possible that some of the positive fixations obtained in clinically negative cases are due to this factor alone.

Prior to carrying out the complement fixation tests for *E. histolytica*, the majority of the sera were submitted to Wassermann or Kahn test or both in the Clinical Laboratories of the Department of Internal Medicine. It was found that existence of syphilis did not affect the specificity of the test. This confirms the previous findings of Craig and also of Spector.²⁸

COMMENT

Hegner²⁹ has recently suggested that there are at least several types among the amebas according to their sites of localizations, each differing in their morphologic and physiologic characteristics, and that the universal conception that the tissue amebas give rise to the lumen types appears to be erroneous. He based his opinion upon histologic studies of the infected intestine which revealed no apparent tissue invasion. Judging from these observations, the formation of antibodies in response to the parasitic invasion may be variable according to the type of the amebas in question. It may be that antibody response is lacking in the case of the lumen and stool types, and this may explain the single instance reported above in which there was a negative complement fixation reaction with a positive stool.

Craig³⁰ has emphasized the fact that carriers, although showing no apparent symptoms, nevertheless usually presented definite pathologic lesions in the intestines at autopsy. It is probable that this explains the positive results in the complement fixation reactions in the 8 cases reported in this paper.

SUMMARY

1. A new culture medium (S. C. medium) for *E. histolytica* is described. By virtue of its simplicity and apparent efficiency, it is suggested that cultures in this medium may be valuable as a part of the routine diagnosis of infections with *E. histolytica*. The culture medium when superimposed upon Dorsett's egg slant serves very well for primary and subcultures. The fact that in these mediums other endamebas fail to grow seems to be of decided advantage in diagnosis.

2. When complement fixation tests were carried out with an antigen prepared from amebas cultured in the above medium, the following results were

obtained: Of 153 individuals examined, 135 known to be free of amebic infection, all were found to give a negative serologic test. Of the remaining 18 cases, 8 were known carriers of *E. histolytica* and gave positive serologic reactions. Six were diagnosed as clinical amebic dysentery and exhibited positive serologic reaction except in one case in which repeated trials failed to show any capacity to fix complement. There were 4 cases of ulcerative colitis which showed positive serologic tests, but no amebas were detected in the examinations of stools.

Thus, a negative serologic test appears to be quite significant, and valuable for eliminating the possibility of amebiasis in a given individual. A positive serologic test also seems to be quite specific in demonstrating the presence of *Endameba histolytica*.

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A STUDY OF THE SALIVARY AMYLASE IN PATIENTS WITH PERNICIOUS ANEMIA*

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PATIENTS with pernicious anemia are known to be subject to glossitis and often have involvement of the buccal mucous membrane. Since the ducts of the salivary glands open into the oral cavity, it was felt that it would be of interest to determine the amylolytic activity of the saliva, in order to ascertain whether the salivary glands were involved similarly. Carles and Delmas-Marsalet,¹ in 1924, reported that the amylolytic power of the saliva was lowered in cachectic states, such as tuberculosis, and increased in patients with peptic ulcer, indicating a possible parallelism with the gastric juice acidity.

The gastric juice findings of the patients presented in this paper have been previously reported by Helmer, Fouts, and Zerfas.² All of the patients had an achylia gastrica after histamine stimulation and were clinically and hematologically typical of pernicious anemia. Healthy adults with normal gastric juice acidity after histamine stimulation were used for controls.

METHOD

The method employed consisted essentially in the digestion of a starch solution with saliva diluted to a suitable concentration and the determination of the glucose formed by the resulting hydrolysis. Results, satisfactory from the comparative standpoint, were obtained, using a substrate consisting of 5 c.c. of a 1 per cent starch solution, unbuffered, and containing no added activating substances. Samples of saliva were collected a minimum of two hours after the ingestion of food or sweets, the flow of salivary secretion having been stimulated by the chewing of paraffin. The saliva was then filtered and diluted to a concentration such that 1 c.c. of the solution incubated with the substrate for one-half hour at 40° C. would hydrolyze between 0.4 mg. and 1.5 mg. of

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glucose. The glucose content of the resultant mixture was determined by the revised method of Folin.²

The limits of true proportionality (0.4 mg. and 1.5 mg.) were determined experimentally by the digestion of 1 per cent starch with 1 c.c. samples of the same saliva diluted in the following proportions with water: 0.4 per cent, 0.6 per cent, 0.8 per cent, 1.2 per cent, 1.6 per cent, and 2.0 per cent. The amounts of glucose that were formed on hydrolysis are shown in Table I.

TABLE I

THE AMOUNT OF GLUCOSE PRODUCED BY DIFFERENT DILUTIONS OF THE SAME SALIVA

SAMPLE	PER CENT SALIVA	MG. GLUCOSE
1	0.4	0.41
2	0.6	0.50
3	0.8	0.68
4	1.2	1.01
5	1.6	1.41
6	2.0	2.06

Apparently the small amount of reducing substances ordinarily present in soluble starch had little influence on the resultant values, at least so far as a comparative effect was concerned, within this range, and it was unnecessary to consider a blank determination inasmuch as the same starch was used throughout.

Since the salivary amylase is so active, cotton plugs were placed in the pipettes to prevent contamination. The water-bath was carefully regulated, and the incubation was carefully timed from the addition of the enzyme to its destruction by the Folin-Wu copper sulphate reagent. The starch solution deteriorates rapidly at room temperature, so it was either made up daily or preserved with toluene, which did not interfere in the determination.

TABLE II

THE AMYLOLYTIC ACTIVITY OF THE SALIVA OF PATIENTS WITH PERNICIOUS ANEMIA AND OF NORMAL CONTROLS

	CASE	DILUTION OF SALIVA	MG. GLUCOSE	UNITS
Pernicious anemia	1	1.0%	1.61	161
	2	0.5%	0.65	120
	3	0.1%	0.75	750
	4	0.5%	0.68	136
	5	0.5%	1.04	208
	6	0.2%	1.24	620
	7	0.4%	0.65	162
	8	1.0%	1.68	168
	9	1.0%	1.17	117
	10	0.1%	0.67	670
	11	0.3%	1.22	406
	12	0.5%	1.31	262
	13	0.5%	1.25	250
	14	0.5%	1.42	284
Normal	1	0.5%	0.80	160
	2	0.5%	0.73	146
	3	0.5%	0.92	184
	4	0.3%	1.57	525
	5	0.5%	0.58	116

For means of comparison, a unit of activity was adopted, namely, the quantity of enzyme necessary to hydrolyze 1 mg. of glucose under the conditions described.

RESULTS

The results of the analysis are shown in Table II. Although the values varied enormously among different individuals, determinations on the same subject over periods of weeks demonstrated but slight variations. These results would indicate that the amylolytic activity of the saliva was not decreased in patients with pernicious anemia. Since all of these patients had an achylia gastrica, the degree of acidity of the gastric juice bore no relation to the activity of the salivary amylase. Furthermore, since patients with pernicious anemia have no free hydrochloric acid in their gastric juice, a considerable amount of starch digestion could take place in the stomach.

SUMMARY

1. A technic is described suitable for the quantitative determination of salivary amylase.

2. No demonstrable deficiency could be found in salivary amylase in patients with pernicious anemia.

The authors wish to express their thanks to Doctor L. G. Zerfas for his kind interest in the work.

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SICKLE CELL ANEMIA*

A CASE REPORT

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THE importance of careful routine laboratory procedures is often minimized. In the case to be reported, the diagnosis, uncertain at first, and the explanation of several obscure physical findings, were at once made clear by the study of the stained blood smears taken by routine. Fradkin and Schwartz¹ say: "It is only by the accidental discovery of the sickle cells in the routine examination that the diagnosis is made." The word "accidental" should be unnecessary.

A brief review of the usual symptoms and findings in sickle cell anemia includes the following: (a) sickling of the red blood corpuscles; certain and varying numbers of the red blood corpuscles assuming a crescentic shape; (b) anemia, the hemoglobin usually averaging about 50 per cent and the total red blood corpuscles about 2,500,000 per c.c.; (c) the liver usually enlarged; (f) spleen, at first enlarged, a little later becomes fibrotic and smoother than normal; (g) trophic ulcers constantly seen; (h) heart murmur; (i) abdominal pain. Alden says the last is found in eight out of eleven published cases. while Hein and McCalla² list abdominal pain as the chief complaint.

Little is known as to the etiology of this peculiar disease. Eastland and Higgins⁴ summarized the ideas of various workers, placing the blame for the changes in the morphology of the blood on: (1) primary faulty erythropoiesis;⁵ (2) surface tension phenomena;⁶ (3) immediate action of toxic, metabolic, or infectious agents upon a racial characteristic;⁷ (4) changes in plasma;⁸ (5) anoxemia.⁹ The mechanism and the etiology remain obscure.

Since 1923 over 100 reports of sickle cell anemia have appeared in the literature. In many reported cases the sickle cell anemia was a secondary manifestation and the cause of death was certainly not the anemia. In some of these cases treatment failed to change the anemia, while the symptoms improved. Examination of healthy negroes showed as many as 16 per cent with the sickling tendency but without anemia. A study of autopsy material from the negro race by Rich¹⁰ showed 62 instances of sickling in 5,000 autopsies, in none of which was the disease suspected. Furthermore, the sickling tendency does not seem peculiar to negroes, since Cooley and Lee¹¹ describe it in a family of Greeks.

Lawrence¹² reports three cases, not all negroes, in which the morphologic changes in the red blood cells were found but in which the classical picture was not so obvious. Recently, Terry, Hollingsworth and Eugenio¹³ presented

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more than 50 cases showing elliptic erythrocytes without any relation to age, sex, color, or blood group, and without relation to sickle cell anemia. A complete bibliography is given by Fradkin and Schwartz. The blood findings and the physical findings so often seen offer an interesting field for further study.

The treatment of sickle cell anemia is unsatisfactory. In spite of the therapeutic measures used, the disease usually proves fatal; few of the patients in the reported cases reaching thirty years of age. Among the procedures, splenectomy has been done with varying results. The use of splenectomy is based on the hypothesis that the red blood cell destruction takes place chiefly in the spleen, and upon the results in hemolytic jaundice. However, this procedure gives no assurance of help, although it tends to relieve the excessive red blood cell destruction. Stewart,¹⁴ Bell and his associates,¹⁵ Cooley and Lee,¹¹ and Hahn,¹⁶ have reported cases in which splenectomy was done without cure but in which the excessive red blood cell destruction was lessened. Transfusions have been tried and invariably give only temporary relief. Liver therapy does not give a satisfactory response. The reasonable treatment consists of such supportive measures as generous amounts of wholesome food, rest, fresh air, and avoidance of infection, with blood transfusion and iron and arsenic to combat the anemia. Splenectomy should perhaps be advised as a last resort.

The differential diagnosis is sometimes difficult. Alden comments that tertiary syphilis obscured the diagnosis for months in one of his cases and that acute appendicitis was strongly suggested in another. Smith¹⁷ reported a case in which congenital hemolytic jaundice was the tentative diagnosis. The enlargement of the heart with murmur often leads to the diagnosis of endocarditis. Probably the symptoms and findings most commonly suggest congenital hemolytic jaundice. However, in this latter condition leg ulcers and joint pains are rare, and the spleen is always enlarged. The laboratory findings of sickle cells and a normal fragility of red blood cells confirm the diagnosis of sickle cell anemia. Syphilis is readily ruled out with serologic tests. The diagnosis rests upon consideration of the blood picture, and the typical physical findings.

CASE REPORT

J. H., a negro boy, aged ten years, was admitted to the services of one of us, with a complaint of chronic ulcer of the leg. The ulcer was situated over the left external malleolus and had been present for over a month. There was no history of trauma. In the past this boy had been considered "delicate." He could not play with other boys of his own age because of dyspnea and shortness of breath. He had no illnesses but mumps in early childhood. No family history relating to his present illness could be elicited.

Only the positive findings on physical examination will be recorded. His sclerae showed a moderate jaundice. The heart was markedly enlarged to the left, and there was a loud systolic murmur heard over the mitral area, accompanied by a thrill. The liver was enlarged four fingerbreadths below the costal margin, and the spleen was not palpable. There was a small ulcer over the left external malleolus measuring 7 cm. by 4 cm., with its long axis vertical. The ulcer showed a granulating, unhealthy base covered by exudate. The edges were slightly undermined.

THE LABORATORY FINDINGS

Urine.—In several dozen specimens albumin was constantly present. Casts appeared at intervals and pus was always present.

Blood Count.—The red blood cells ranged from 1,500,000 to 2,260,000, and the hemoglobin remained about 45 per cent. The greatest improvement was seen temporarily following transfusion. Morphology of the red blood cells showed many "sickle" cells (Fig. 1) in both wet and stained preparations. This picture remained constant throughout the disease. Poikilocytosis and anisocytosis were always noted. The white blood cells totaled always about 10,000, a terminal count showing 27,000. The polymorphonuclear cells varied as the total white blood cells. Fragility tests showed a normal range. Bleeding time was one and one-half minutes, coagulation time two minutes, icteric index 20, and the van den Bergh a positive (faint) delayed direct reaction. Wassermann and Kahn reactions were negative. Blood cultures showed no growth. Blood chemistry: nonprotein nitrogen 66 mg.; creatinine, 1.3 mg.; sugar, 90 mg.; and chlorides, 460 mg. Blood group of the patient, father, and mother were (Moss) Group IV. Cross match was without change with the father. The father had a negative Kahn test.

Throat cultures were negative on two occasions. Stool negative on four occasions.

X-ray showed enlargement of heart to the left. Lungs and mediastinum were negative.



Fig. 1.—Showing the "sickle cells."

PATHOLOGIC REPORT

Gross.—Specimen consisted of a spleen, a small lymph node, and a piece of liver. The spleen was moderately enlarged and of the usual shape. It was firm in consistency and considerable fibrous tissue was noted throughout. Here and there were round, yellowish nodules of a soft consistency. The whole spleen was purple, mottled with a large amount of yellow. The lymph node measured only $1\frac{1}{2}$ by 2 cm. and was soft and of a purple color. The small piece of liver showed nothing in the gross except that it was soft and red.

Microscopic.—Sections of spleen showed a thick, fibrous capsule. There was a large infarct, the center of which was filled with red-staining, necrotic material. Its margins were definitely fibrous and filled with chronic inflammatory cells. The pulp of the spleen was packed with red blood cells. In the interspaces were many "sickled" cells. Sections of liver showed areas of dilatation of the blood spaces. Here also were many "sickled" red blood cells. The liver cells showed everywhere clouding and granular changes in their cytoplasm. The interstitial tissue was moderately increased, and filled with chronic inflammatory cells. Sections of lymph node showed sinuses filled with blood, including many sickle cells. The follicles were small and widely separated.

Diagnosis.—White infarct of spleen, splenomegalia (sickle cell anemia), chronic hepatitis, chronic congestion of liver, hyperplasia of mesenteric lymph nodes, and chronic lymphadenitis.

TREATMENT

The treatment consisted of general measures, as rest in bed, sunlight, and nutritious food. The ulcer was treated by elevation of the part, and wet dressings, with no response. A transfusion, using the father as donor, was done. After several days some improvement in the boy's general condition, and in the anemia was noted. This improvement was only temporary and on the thirtieth day, after consultation, it was decided to do a splenectomy. The operation was uneventful, the spleen being removed without difficulty, and a wedge of liver taken. Unfortunately, postoperative pneumonia on the third day was followed by the death of the patient. Every effort to obtain an autopsy was combated by the parents.

SUMMARY

A brief review of the recent literature on sickle cell anemia is given and the outstanding symptoms and findings are outlined; the etiology being reviewed from the literature.

Sickle cell anemia being a rare and obscure disease, the treatment is largely symptomatic, and seldom successful.

The diagnosis may be made without difficulty. Too much emphasis cannot be placed upon the careful routine examination of the blood, which should include wet preparations as well as stained smears.

The case presented shows all the characteristic findings of sickle cell anemia. Transfusions and splenectomy failed to cure the patient.

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THE MECHANICS OF SPLEEN VISUALIZATION BY MEANS OF METALLIC COMPOUNDS, IN PARTICULAR THORIUM*

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SINCE thorium compounds such as "Thorotrast" are being increasingly used for spleen and liver visualization, we believe that all possible information as to their action and effects should be made available. Following the original reports of Radt¹ and Oka,² on the use of a colloidal thorium compound for spleen and liver visualization, we began some experimental work early in 1931 in an attempt to arrive at the exact method of visualization. The reports of these workers and R. Jaffe³ gave the impression that the reticuloendothelial cells of organs, such as the spleen and liver, took up the thorium; which element was therefore directly responsible for the x-ray visualization that was obtained.

We used approximately the same thorium preparation as these authors had suggested. The method of preparation was essentially that described by Bluhbaum, Frik, and Kalkbrenner, quoted in detail by Radt. The actual preparation of our thorium compound was done by Dr. Marberg, a biochemist at the University of Chicago. It was prepared as follows:

Freshly precipitated thorium hydroxide, prepared from 20 gm. of thorium nitrate, was washed free of electrolyte, suspended in 100 to 150 c.c. of water. The hydrosol was peptized by the addition of small amounts of normal hydrochloric acid added to the boiling suspension over a period of five hours. (Note: The use of the hydrochloric acid is a modification by Dr. Marberg, and we believe it makes a less toxic final production.) The final solution (150 c.c.) contains 20 gm. of thorium nitrate as $\text{Th}(\text{OH})_4$. This is equivalent to 9.78 gm. of thorium or 12.5 gm. of thorium hydroxide. Also equivalent to 0.0653 gm. of thorium per c.c., or 0.0833 gm. of thorium hydroxide per c.c. The solution contains 0.0643 mols of hydrochloric acid per liter. Stable at boiling and refrigerator temperatures; also stable toward addition of neutral salts and glucose.

A dog two weeks old weighing 1.4 kilo was x-rayed and showed no spleen shadow. On Feb. 21, 1931, 1 c.c. of thorium solution, plus 7 c.c. of normal saline, was injected into the anterior fontanel with no clinical ill effect. A film taken twenty-four hours later showed faint visualization of the spleen. There was excellent visualization at forty-eight hours. At ninety-six hours the spleen shadow was beginning to fade. The liver was possibly denser than the other upper abdominal viscera, but could not be definitely differentiated. On Feb. 27, 1931, the same dose of thorium solution was repeated at the same site. Thirty hours later the spleen visualization was again at its maximum. The animal was killed and autopsied Feb. 28, 1931.

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Microscopic study of sections of the injected spleen showed irregular degeneration and cloudy swelling of its cells, especially adjacent to the sinuses and capillaries, the latter were crammed with thorium particles, and discrete brownish particles were scattered diffusely throughout the stroma. The corpuscles were relatively unaffected. There seemed to be no tendency for the ingestion of the particulate matter (thorium) by the reticuloendothelial cells.

We next used three dogs about two months of age but varying considerably in size. Dog 1 was a control, weighing 2,200 gm. The other dogs weighed 1,100 and 1,600 gm. respectively. All were x-rayed and showed no evidence of a splenic shadow. One cubic centimeter of a special colloidal solution of mercuric sulphite was administered intravenously to the 1,100 gm. dog. Films were taken twenty-four hours after injection and again at forty-eight hours; the spleen was not visualized. The 1,600 gm. dog was given 1 c.c. of the thorium solution plus 4 c.c. of a mixture of 10 per cent glucose and normal saline by the anterior fontanel. There was no systemic reaction. In twenty-four hours there was excellent visualization of the spleen, the shadow remaining the same density at forty-eight hours. All three dogs were killed within fifty hours from the beginning of the experiment. At autopsy the only gross change observed was in Dog 2 (the one injected with mercuric sulphite) whose spleen showed a purplish black discoloration.

Microscopically the lymphoid cells of the control spleen were normal. There were a few particles of hemosiderin here and there, but they were not dense.

The spleen of the "mercury" Dog 2 showed the cells crowded together with some swelling and pallor. There were much larger pigmented particles than the hemosiderin throughout the sections, but none of the large particles (mercuric sulphite) were inside the cells of the reticuloendothelial type. The blood vessels were filled with leucocytes and in many places contained much of this particulate material. In the spleen from the "thorium" dog there was swelling of the stroma and the cells were pale and swollen. The blood vessels showed the same type of leucocytic chemotaxis as in the "mercury" dog. The Malpighian corpuscles were not appreciably involved. No thorium had been taken up by reticuloendothelial cells but the stroma was literally peppered with thorium particles. A few macrophages were present and had ingested various quantities of thorium. Those that contained the greater quantity of thorium showed a marked degeneration.

It seemed from the above that simple cloudy swelling might prove to be an essential factor in visceral visualization of this type. Therefore, we undertook to study it in vivo. We gradually poisoned a three-month-old dog with a soluble salt of mercury (bichloride of mercury), giving $1\frac{1}{2}$ gr. a day for three days, 7 gr. on the fourth day, and 7 gr. intramuscularly on the fifth. The spleen was visualized by x-ray on the fifth day quite clearly, and also on the sixth day, but perhaps a little less distinctly. The viscera as a whole seemed to show more definitely than usual. The dog was killed on the sixth day when acutely ill with mercurial nephritis.

Microscopically the spleen showed lymphocytes that were unusually large and crowded together. In fact the cells were so pale and swollen that it was difficult to differentiate lymphocytes from reticuloendothelial cells, monocytes, and etc.

RÉSUMÉ

The method of Oka and Radt for visualizing the spleen by the administration of thorium intravenously seems to be adequate as far as x-ray findings are concerned. However, the thorium seems to produce an intense degeneration of the spleen in doses too small to give clinical signs. The thorium appears to be taken up largely by the blood sinuses and lodged in the stroma rather than in the reticuloendothelial system. Other colloidal particulate matter (mercuric sulphite) is taken up in the same way but does not produce x-ray visualization of the spleen apparently because cloudy swelling does not result.

The spleen is visualized if an acute cloudy swelling is produced as in bichloride of mercury poisoning, but this visualization is not as marked as that obtained with thorium.

CONCLUSIONS

1. Intravenous injections of thorium give satisfactory x-ray visualization of the spleen.

2. This visualization of the spleen is due in part to the thorium particles in the stroma and capillaries, but cloudy swelling seems to be also an important factor.

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A STUDY OF PUNCTATE STIPPLING AS FOUND IN THE LEAD POISONING OF WILD DUCKS*

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PUNCTATE or basophilic stippling of erythrocytes has apparently been a subject of controversy since it was noted by Ehrlich in 1885.¹ Stippling was first correlated with lead poisoning by Behrend some fourteen years later, in 1899.² A voluminous literature on the subject accumulated, which when summarized by Pappenheim³ in 1919, fell far short of proving the mechanism of its production, or of a clear-cut clinical conception of its significance. The toxic conditions in which stippling was found were well known; but regarding the granules themselves, opinions varied from the toxic granular degeneration theory of Pappenheim to the nuclear fragmentation theories of Kreibich⁴ and Koch.⁵ Many of our standard textbooks still quote one or the other of these theories, which, in fact, are both undoubtedly wrong.

Modern knowledge regarding this phenomenon began with Key in 1924.⁶ Working with rabbits in experimental lead poisoning he proved that the percentage of stippled cells rose and fell in direct proportion to the reticulocyte variation, and that the number of reticulocytes was approximately equal to the combined number of stippled and basophilic erythrocytes, thus proving that stippled cells were immature erythrocytes. Four years later Brookfield⁷ confirmed this finding in human subjects.

Cooke and Hill⁸ in a study of the microscopic picture of pernicious anemia state that stippling is produced by the remains of the original cytoplasm of the erythroblast; and that the reason for its taking the azur-eosin stain after fixation with alcohol may be found in a defect in the immature lipin covering of the cells. Lane⁹ shortly thereafter agreed with Cooke's hypothesis and further disproved the old theories by an ultramicroscopic (dark-field) study of stippled cells, the granules of which could not be demonstrated in wet or wet-fixed films. As all other toxic granular degeneration products or nuclear fragments can easily be demonstrated by strong indirect illumination, it seems thoroughly proved that the accumulation of basophilic staining material into clumps in certain young erythrocytes represents an alteration of the protoplasm of these cells that interferes with its ability to remain diffused throughout the cell during the process of drying.

Incidental to a survey of wild fowl in Louisiana to determine the occurrence and extent of accidental lead poisoning from ingested shot, a careful blood survey was added to the routine pathologic examination. Numerous specimens showing all degrees of stippling were encountered. The presence of nucleated erythrocytes in fowls enables a direct determination of the degree of poisoning

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necessary to produce stippling. The occurrence of basophilic stippling in large numbers of ducks is also of interest in view of the statement by Key⁶ that several investigators, including A. S. Minot, had been unable to produce it in chickens.

Wild duck arriving in southern Louisiana in mid-November congregate on large shallow lakes formed by inundation of former agricultural reclamation projects in the fertile marsh areas. Members of the seaup group, particularly black duck and d'eau gris, in feeding on the seeds of an aquatic plant which are about the size of ordinary lead shot, find and ingest large numbers of lead pellets



Fig. 1.—Normal blood of wild duck.

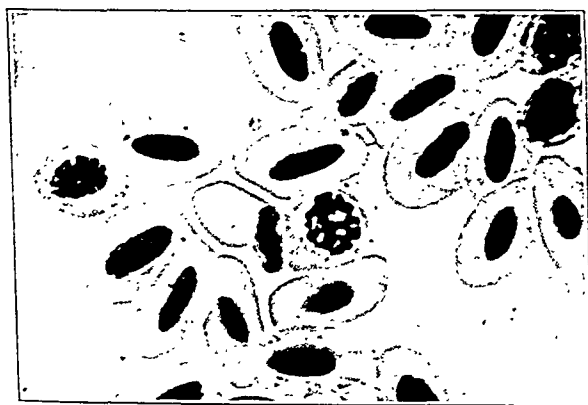


Fig. 2.—Active blood regeneration following acute blood loss in acute lead poisoning. Numerous shot present in the gizzard that had lost only a fraction of their lead content.

that have accumulated during the past few years of intensive hunting. Wetmore¹⁰ presents a clinical and experimental study of this lead poisoning, citing experiments proving that three or four No. 6 shot will usually kill a captive wild duck. An average incidence of 12 shot were found in 89 ducks of this species in this investigation, the individual variation being from 0 to 140 shot.

Duck arriving in the ponds relatively free from lead (see Fig. 1) acquire their lead in apparently a few days' feeding. Within a week, and while still able to be taken on the wing, they present all of the symptoms of an acute lead

poisoning (Fig. 2) with tremendous loss of weight, anemia, diarrhea, bile-stained mucosa of the gizzard, active hemopoiesis of the long bones of the leg and an enormous increase in the number of reticuloocytes, which may reach a total of 70 per cent of the cells in the peripheral circulation, together with large numbers of erythroblasts and megaloblasts. Those that survive this acute poisoning, as shown by the presence of numerous shot in the gizzard, and which have lost from 20 to 50 per cent of their former weight, now begin to show numerous stippled cells (Fig. 3) coincidental with unmistakable changes in the nucleus, indicating cell death. The nuclei of many of the slightly basophilic erythrocytes become swollen and pale when stained with Wright's stain. Stippling appears, the nucleus changes position to one side or one pole of the cell. In those cells with the largest basophilic granulation, the nucleus appears only as a shadow. Fragmentation of the nucleus can be observed as a terminal process in these stippled cells, the nuclear fragments staining a definite purple and the basophilic granules a deep blue with the polychrome stains. It would seem reasonably certain that the direct combination of lead with phosphates on the

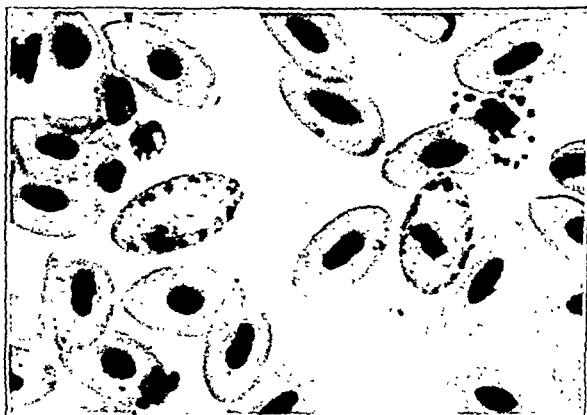


Fig. 3.—Chronic lead poisoning with stippling and nuclear degeneration and fragmentation. Numerous shot present in the gizzard that had been ground down to show a loss of 3 or more grams of metallic lead.

surface of the erythrocytes and the local liberation of a weak acid, as shown by Aub, Reznikoff and Smith,¹¹ is sufficiently toxic to produce actual cell death. The original cell protoplasm, represented by the basophilic stain of the erythrocyte, is not then able to withstand even the momentary effect of still further injury in drying, but precipitates or condenses into a granular form. It is thus demonstrated that the significance of a stippled cell is that of a dying cell and not merely a young cell that may continue to mature in the circulation. This visual proof of the chemical selection and untimely destruction by lead salts of a semimature cell is direct proof that stippling is a pathologic change in the sequence of events in the usual maturation of such cells in the peripheral circulation.

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LABORATORY METHODS

A NEW SYPHILIS REACTION, THE M.K.R. II IN CEREBROSPINAL FLUIDS*

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THE procedure for using the M.K.R. II in *serum examination* as now widely practiced may be briefly summarized as follows: 0.2 c.c. active serum is mixed with 0.5 c.c. of a freshly prepared suspension of the blue M.K.R. II antigen, and the test tubes are well shaken. For reading the results there is a choice of four different indicators:

1. *Macroscopic flocculation reaction*: The flocculation is read like an agglutination test after one and one-half hours with a hand lens.

2. *Microscopic flocculation reaction*: Immediately after setting up the tests drops of the reaction fluid are taken with a pipette and deposited on level slides which must be kept in a moist chamber for one hour, whereupon the reaction is read under a microscope with sixty fold magnification.

3. *Clarification reading*: The test tubes are left overnight at room temperature and the degree of clarification in each tube is then ascertained with the naked eye.

4. *Centrifugal technic*: Immediately after setting up the tests, the test tubes are centrifugalized for ten minutes. When they are taken out of the centrifuge, the supernatant fluid is carefully poured off and the tubes placed upside down in a rack. They are examined in half an hour's time to see whether the sediments have run down the sides of the tubes, or whether they have remained unaltered.

In the course of *experiments with cerebrospinal fluids* which I carried out jointly with Dr. Holthaus, it soon became apparent that the dosage of the serum test and the various methods of reading it could not be applied without some modification to the investigation of spinal fluids. Weak positive spinal fluids, in the dosage 0.2 c.c. fluid + 0.5 c.c. antigen suspension, were found to give a negative reaction. Thereupon we increased the amount of fluid, at the same time decreasing the amount of antigen, for instance: 0.5 c.c. spinal fluid + 0.1 c.c. antigen suspension. It now showed itself however that, owing to the small amount of antigen, the reaction fluid was not turbid as in the serum test, but more or less clear from the outset, so that there could be no question of applying the clarification indicator to the readings. But neither were the flocculation reading or the centrifugal technic quite satisfactory.

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We were therefore under the necessity of searching for a new indicator for *spinal fluid examination*; this we eventually found in *sediment reading*. If, after setting up the tests, the test tubes are left standing overnight at room temperature, a deposit of sediment becomes visible in each tube presenting variations in the case of negative or positive reactions which are easily recognizable with the naked eye. In *negative* reactions a blue, fairly clean-cut disc-shaped sediment forms at the bottom of the tube. In *positive* reactions there is no sign of this blue disc, but the bottom of the tube is covered with a light, whitish, filmy sediment, sometimes so slight as to be only distinctly ascertainable as sediment with the help of a hand lens. *The determining factor for the appreciation of the reaction is always the presence or absence of the characteristic blue, disc-shaped sediment. If this is visible, the reaction must be marked negative; if not, the result is positive.*

TECHNIC OF THE SPINAL FLUID REACTION

1. *Reagents*.—The same M.K.R. II Standard Antigens are used as for the serum tests. Also the 3.5 per cent salt solution of the serum tests is used in this reaction; for this an absolutely pure preparation is necessary, viz.: natrium chloratum pro analysi.

2. *Pipetting the Spinal Fluids*.—Small test tubes about 8 cm. in length and as exactly as possible 1 cm. in diameter (so-called M.K.R. tubes) are used; they should be uniformly well rounded at the base. The tubes are placed in a perforated tube rack. Care must be taken that the perforations in the lower ledge of the rack be of a size which, while preventing the test tubes from falling through, leaves as much as possible of the surface of the bottom of the tubes open to examination from beneath through the holes in the rack.

The *spinal fluids* must be examined in an *active* state. It is absolutely essential that they are *thoroughly centrifugalized* so as to be entirely free of any corpuscular admixture. When the spinal fluids are sent to the laboratory from a distance, it is most important that the containers should *not be closed with cork, but with rubber stoppers*. Nonspecific reactions may sometimes be occasioned by the dissolving of certain substances during the transport when cork stoppers have been used.

Each spinal fluid is operated in 3 tubes: in the first, 0.5 c.c. fluid, in the second, 0.2 c.c., in the third, 0.1 c.c.

3. *Preparation of the Antigen Suspensions*.—The requisite quantity of standard antigen* for the tests is pipetted to the bottom of a large test tube

PREPARATION OF THE M.K.R. II STANDARD ANTIGEN

**Heart Powder*.—The antigen is made from perfectly fresh beef hearts. The hearts are freed from fat and sinews, so that the pure heart muscle is left. This is put through an ordinary meat mincer, and then the finely chopped-up heart muscle is spread out in a flat layer on glass dishes; these are placed in a drying apparatus at from 40 to 50° C. The drying is continued until the heart pulp separates from the glass in hard crusts. These crusts are completely scraped off the glass dishes with a knife, and then placed in the incubator at 37° for two days, in order to secure its being dried as thoroughly as possible. The relatively large hard crusts of the dried heart muscle are reduced to small pieces in an absolutely dry meat mincer, and are then put through a coffee mill, so that an extremely fine powder results. This is poured into dry flasks which should be well stoppered and kept in a cool room. In making the antigens, it is advisable to mix the powder of several different hearts, because the individual hearts show slight differences in their lipid content.

Ether Extraction.—Put 10 gm. of the heart powder into an Erlenmeyer flask of 100 c.c. content, or into a bottle of the same size. The glass vessel must be closed with a stopper covered with tin foil of good quality. Extract the powder first with ether and afterward with alcohol. Use exactly 70 c.c. ether to 10 gm. of powder; shake it up several times, and let the

or pointed glass vial. The tenfold quantity of 3.5 per cent salt solution is put into a second tube or vial of the same size. Antigen and salt solution are heated *separately* in a water-bath at from 57 to 58° until they have reached a *temperature of 55-56° C.* (about 10 minutes). The right temperature may be easily ascertained by means of a thermometer which has been placed at the same time in the water-bath in a vial filled with ordinary water. The level of the water in the water-bath must be 1 or 2 cm. higher than that of the salt solution in the vial in order to ensure uniform heating. If there is not a regulated water-bath at disposal, an ordinary tub may be filled with water at from 60 to 62° C. and, when the vials and control glass have been put in, left to cool down naturally to about 57° C.

After this preliminary heating of the reaction fluids, the salt solution must be poured rapidly into the vial containing the antigen, the mixture then poured back into the empty glass and back again into the antigen glass. *The mixing must be executed rapidly*, but care must be taken to avoid spilling any of the fluid during the manipulation. The freshly prepared antigen suspension is of a milky turbid bluish hue; it must be put back *at once* into the water-bath for *exactly two minutes* for *maturing*, and then utilized immediately for the tests.

In most laboratories the examination of spinal fluids will be only a fraction of the serum examinations. The antigen suspensions for sera and spinal fluids being identical, *the same antigen suspensions are of course used for both*, and it is not necessary to prepare them especially for spinal fluids.

4. *Mixing Spinal Fluid and Antigen Suspension.*—To the first tube with 0.5 c.c. spinal fluid, 0.1 c.c. antigen suspension is added; to the second with 0.2 c.c. spinal fluid also 0.1 c.c. antigen suspension, and to the third with 0.1 c.c.

vessel stand for two hours, shaking several times again during this period. Filter off the ether through an ordinary paper filter, and again add 70 c.c. of ether to the powder. Proceed then in the same way as before for two hours. Filter off the ether once more. For the third time, add 70 c.c. of ether to the heart powder. Shake up several times during the next two hours. Afterward let the vessel stand undisturbed until the following day, and then filter the ether off. On filtering the whole powder is as far as possible, put on to the filter. After the fluid has passed through to the last drop, the filter should be carefully spread out flat and placed in the incubator at 37° until the next day. The glass vessel in which there still remain traces of the powder, should likewise be placed in the incubator. By the following day, the ether will have evaporated for certain. Put the powder on the filter back again into the bottle, and begin with the alcohol extraction.

Alcohol Extraction.—Pour 80 c.c. of 96 per cent alcohol over the heart powder which has been collected as completely as possible in the glass vessel. Let the bottle stand for eight days at room temperature, thoroughly shaking it several times a day. Then filter off and keep the heart extract at room temperature.

Adding of Balsam of Tolu and Victoriablue.—Dilute 10 c.c. of the heart extract with 90 c.c. of 96 per cent alcohol and add 1.4 gm. of balsam of Tolu to this dilution. Place the bottle in the incubator at 37° for two days and shake it several times a day. Then keep it at room temperature for a week and filter off through an ordinary paper filter. After adding of 0.01 gm. of Victoriablue to the filtered Tolu-Antigen it is ready for use.

Standardization of the M.K.R. II Antigen.—The Antigen thus prepared possesses approximately the right lipid content of 1:10, requisite for the syphilis tests. In order to be able to weaken or to strengthen it, as the case may be, some balsam of Tolu in the proportion of 1.4 per cent is dissolved in the undiluted alcoholic extract which is rich in lipoids, and some also in an extract diluted 1:100 with alcohol which is extremely poor in lipoids. The addition of the undiluted extract to the M.K.R. II antigen serves to increase its lipid content and thus to weaken the sensitivity of the tests; the addition of the much diluted extract, to lower the lipid content and thus to make the tests more sensitive.

The standardization of the antigen must be performed, in testing it with positive and negative sera, according to the prescriptions for the M.K.R. II (Zentralbl. f. Bakteriöl. 125: Pt. 1, 1932). In case the results in the serum tests should be somewhat too strong, and there should be a tendency to unspecificity, the lipid content of the antigen must be slightly increased. If, on the contrary, the results are too weak, as compared with other syphilis reactions or with the results obtained with a Standard M.K.R. II Antigen, the lipid content of the antigen in question has to be somewhat reduced.

All antigens should be kept at room temperature (not in the refrigerator) in well-stoppered bottles protected from light. The extract bottles must be stoppered with corks which should be covered with tin foil of good quality. If antigens are forwarded from one laboratory to another in frosty weather, it is advisable, on their arrival, to place them first in the incubator at 37°, and only then to store them at room temperature.

spinal fluid, 0.2 c.c. antigen suspension is added. The racks are well shaken after the addition of the antigen suspension.

If it is desirable to appreciate the quantitative degree of weak and medium strong positive reactions *with great exactitude*, two further tubes may be added to the original three. To this end another tube containing 0.4 c.c. spinal fluid and 0.1 c.c. antigen suspension is inserted between the first and second, and between the second and third a tube with 0.2 c.c. spinal fluid and 0.2 c.c. antigen suspension.

5. *Reading the Results.*—The tests having been left standing overnight (sixteen to twenty-four hours) at room temperature of about 20° C., protected from cold draughts, the results are read with the *naked eye*. The investigator stands in front of a well-lighted window or an artificial source of light above his head, lifts the racks carefully, and holds them before him somewhat above his eye level, at the same time slanting them slightly forward. In this position the apertures of the test tubes are in a slanting direction to the source of light above and the base is toward the investigator.

a. *Negative Reactions:* In all three tubes *blue disc-shaped sediments* are visible in the center of the bottom of the tube, their coloring contrasting sharply with their surroundings. These blue sediments are *soft and have a tendency to dissolve*. If the rack is held in a slanting position for some little time, they will, following the law of gravitation, dissolve themselves into a thin streak. Shape, color and dissolubility of the sediments are the characteristics of a negative reaction.

b. *Weak Positive Reactions:* The first tube with 0.5 c.c. of spinal fluid does not show any blue disc-shaped sediment. The bottom of the tube is of a whitish opalescent hue. On close examination a very light filmy sediment is visible uniformly spread over the bottom of the tube. When held slanting for any length of time, this sediment does not dissolve; it will sometimes change its position as a whole, or it may crumble to pieces. Both the other tubes present the characteristic blue negative discs.

When in the first tube neither a distinct dissoluble blue disc nor the uniformly opalescent whitish coloration at the bottom of the tube is visible, the reaction must be marked *doubtful* (\pm). Then there is generally a rudiment of disc formation to be seen in the first tube with a number of minutest fragments of sediment strewn around it. In such cases the central disc is usually not soluble but adheres more or less firmly to the bottom of the tube.

c. *Medium Strong Positive Reactions:* The two first tubes with 0.5 c.c. and 0.2 c.c. spinal fluid show the positive sediment described in paragraph b., while the third tube has a negative disc.

d. *Strong Positive Reactions:* The negative blue disc is not visible in any of the tubes. They all show the connected whitish sediment of a positive reaction completely covering the bottom of the tube. In correspondence to the relatively larger amount of antigen in the last tube, the sediment in it is somewhat denser than in the first two, and may sometimes be slightly bluish in coloring.

e. *Hyperpositive Reactions:* Occasionally, though rarely, in cases of paralysis and tabes, spinal fluids may be observed which react so strongly that they

present an inhibition in the first or in both the first tubes. In these tubes more or less distinct blue negative discs have formed, while the characteristic broadly spread positive sediment appears in the last tube only. Compared to the weak positive reaction there is therefore in such cases a *reversed scale with the strongest reaction in the last tube*.

f. *Bacterially contaminated spinal fluids*, for instance, such as derive from cases of meningitis or have become decomposed during transport, may occasionally show the *same scale as hyperpositive reactions*. They must therefore be differentiated by a special test. To 0.1 c.c. of the questionable spinal fluid, 0.1 c.c. of a proved negative active serum and 0.5 c.c. of the usual antigen suspension is added. The mixture is well shaken and the reading performed in the way usually practiced in serum tests, viz.: either as macroscopic or microscopic flocculation or on the following day as clarification. *In the serum dilution contaminated spinal fluids always give a plainly negative reaction, whereas hyperpositive spinal fluids give a strong positive reaction.*

THE M.K.R. II AS COMPARED TO OTHER SPINAL FLUID REACTIONS

Our comparative investigations comprising some 600 cases have shown that the M.K.R. II applied to spinal fluids is appreciably superior in *sensitivity* to the Wassermann reaction, and is at least equal to the Kahn standard method and the Müller conglobation reaction.

Also with regard to *specificity* the M.K.R. II in spinal fluids is in no wise inferior to the above-named methods. It is a very simple method, is easily read, and provides the possibility of obtaining a good quantitative gradation of positive reactions with only three test tubes.

The M.K.R. II thus belongs to the group of particularly sensitive specific syphilis reactions in spinal fluid diagnosis as well as in serodiagnosis.

A NEW COLORIMETRIC METHOD FOR THE DETERMINATION OF BILIARY ACIDS IN BODY FLUIDS: WITH A NOTE ON THEIR ALLEGED PRESENCE IN NORMAL BLOOD*

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SINCE the introduction of the Pettenkofer¹ reaction for the recognition of bile acids, many attempts have been made to apply this test for estimating bile acids in blood. As a result of these researches it has been assumed that a positive Pettenkofer reaction given by specially prepared extracts of normal blood is an indication of the presence of bile salts. On this assumption Aldrich and Bledsoe² found that normal blood contains bile salts to the extent of 3 to 6 mg. per 100 c.c., while Charlet's³ average findings are given as 3.5 to 3.8 mg. in normal rat blood.

On the other hand, by using different means of isolation but still using the Pettenkofer reaction, Perlzweig and Barron⁴ as well as Gregory and Pascoe⁵ deny the presence of bile salts in normal blood.

Recently Walker⁶ has called attention to the interesting and important fact that the substance responsible for the color production in the Aldrich and Bledsoe process is cholesteryl oleate. This is hydrolysed by barium hydroxide to barium oleate. The cholesteryl oleate and barium oleate both give a positive Pettenkofer reaction.

From a practical standpoint, however, it cannot be denied that nearly all the methods have the common objection in that the Pettenkofer reaction is employed. A critical study of the quantitative aspects of this reaction has revealed many undesirable features in that: (a) the color reaction is not specific for bile acids, Mylius⁷ finding 15 other substances (including barium oleate) that give a Pettenkofer reaction; (b) the quantitative recovery of bile acids is very variable with errors from 1 to 20 per cent; (c) the red or reddish color varies considerably as to color shade making comparison in the colorimeter exceedingly difficult.

It is obvious from the above results that the Pettenkofer reaction is wholly unsatisfactory for quantitative purposes even with pure solutions of bile acids, and quite unreliable even for qualitative work on body fluids.

A large number of tests have been advanced for the detection and estimation of bile salts in the urine, most of which again employ the Pettenkofer reaction, either direct to the urine, or to the isolated salts. If the reaction is applied direct to urine containing proteins a positive Pettenkofer reaction is always obtained. It hardly needs to be emphasized here that chromogenic substances yield intense colors with sulphuric acid. In the isolation method where the bile salts are precipitated from urine by saturation with magnesium sulphate,

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The studies were carried out in the Bio-Chemical Laboratory, Charing Cross Hospital, Institute of Pathology, by kind permission of Dr. J. Patterson, biochemist to the hospital.

bilirubin is also precipitated along with many other urinary pigments. As bilirubin is almost always present in combination with bile salts, the color produced in the final stage of the method is entirely lost in the dark brown or blackish mass obtained by the charring action of sulphuric acid on bilirubin and other urinary pigments. This process yields but little better results than that of the direct method. It is of great importance in patients treated with drugs which are potential liver poisons to try to obtain early warning of toxic action; again in patients with subicteric tints and fevers of unknown origin a reliable and quantitative method might prove of clinical value. Comparatively little work has been done in this direction, owing, presumably, to the methods being subject to the numerous limitations already mentioned.

The work outlined here was undertaken in order to arrive at a quantitative method for estimating bile salts. Various substances other than bile salts giving a positive Pettenkofer reaction and likely to be present in blood were subjected to the new reaction and found to be completely negative. The list includes cholesterol, cephalin, glycine, lanoline, phospholipines, lecithin, taurin, and oleic acid (present as cholesteryl oleate).

The following is the principle of the method:

The urine is acidified with hydrochloric acid and saturated with solid ammonium sulphate. The bile acids are precipitated completely, along with other urinary pigments. Bilirubin when present is also precipitated. To prevent the precipitated bile salts from adhering to the sides of the boiling tube and stopper, a small amount of protein is first added to the urine before adding the hydrochloric acid and ammonium sulphate. The protein has the effect of "holding" together the biliary acids, and thus overcoming great practical difficulties in that obvious loss of material is prevented as well as rendering time-consuming efforts unnecessary. The precipitate is filtered and washed with a saturated solution of ammonium sulphate to remove any soluble adhering pigments. The filter paper is carefully dried and the bile salts plus the insoluble urinary pigments extracted with absolute alcohol. A saturated solution of barium hydroxide is added to the extract, and after further concentration the mixture is diluted to a convenient volume with alcohol, and filtered after thorough cooling. The addition of the barium hydroxide renders bilirubin and other interfering urinary pigments insoluble in absolute alcohol. The alcoholic extract is evaporated to dryness and a reddish purple color produced from the residue by the action of levulose and pure hydrochloric acid. The standard is an alcoholic solution of sodium tauroglycocholate and is similarly treated. The colors are then compared colorimetrically.

Reagents and Apparatus.—

1. Pure concentrated (A. R.) hydrochloric acid (sp. gr. 1.16) $\text{HCl} = 36.465$.
2. Pure syrupy (A. R.) phosphoric acid (sp. gr. 1.75) $\text{H}_3\text{PO}_4 = 98.051$.
3. One per cent pure levulose (Merck) in a saturated solution of benzoic acid. This keeps indefinitely.
4. Saturated aqueous solution of ammonium sulphate.
5. Solid ammonium sulphate.
6. Fresh blood serum or plasma. For the precipitation to succeed it is absolutely essential that the serum or plasma employed be perfectly fresh, and this is usually obtainable in any modern laboratory.

7. Absolute alcohol (97 per cent).

8. Filter papers: Whatman extraction thimbles, size about 22 by 80 mm., and ordinary No. 1 papers, 7, 9, and 11 cm. in diameter.

9. A saturated aqueous solution of barium hydroxide.

10. Five-tenths per cent sodium tauroglycocholate in absolute alcohol. Owing to the hygroscopic nature of the bile salts, it is important to weigh out the above amount on glazed paper, and as quickly as possible. Transfer the bile salts to a 100 c.c. flask, add about 60 c.c. of absolute alcohol and immerse the flask with constant agitation in a boiling water-bath until the bile salts have dissolved completely. Cool, and dilute to the mark with absolute alcohol. After mixing thoroughly, filter through a 9 cm. paper.

11. Preparation of asbestos mat. Use a Gooch crucible of about 15 c.c. capacity (see Fig. 1) or an asbestos mat supported on a small (15 mm. diam.) perforated porcelain plate, resting in a conical funnel that passes through a rubber stopper fitting the neck of a filtering flask. The mat is prepared as follows: A suspension of well-washed asbestos is poured into the Gooch crucible or onto the plate and allowed to settle down without suction. After a short time the filtering flask is connected to the suction pump and the water pressure turned on. It is important not to suck too hard. The amount of asbestos required is such as to form a mat about 3 mm. in depth.

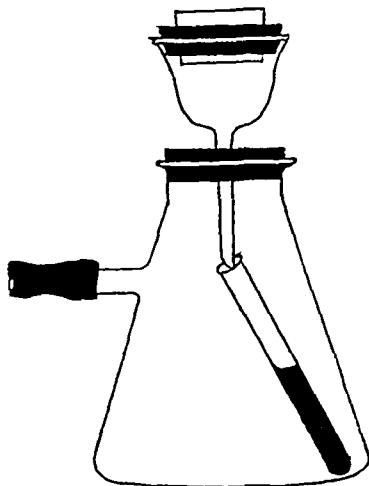


Fig. 1.

A small porous plate may be placed on top of the mat to prevent the latter from being disturbed too much by pouring on water or the phosphoric acid mixture. The mat is then washed two or three times with distilled water, gentle suction being applied after each addition. The final suction should be sufficient to make the mat quite firm. After being used for filtration of the phosphoric acid mixture the asbestos should be washed well with distilled water before further use.

METHOD

For Urine.—Introduce 25 c.c. of urine into a thick-walled boiling tube (Monax combustion tubes, 1 inch by 6 inches) followed by 0.05 c.c. of fresh blood serum or plasma. After mixing with the protein solution, add 2 c.c. of pure concentrated hydrochloric acid, mix again, and then saturate the acidulated solution by the addition of 25 gm. of solid ammonium sulphate. Securely stopper the tube, and after thorough shaking, stand for about ten minutes with occasional agitation to ensure for complete saturation with the ammonium

sulphate. Filter through a Whatman No. 1, 11 cm. paper which has been previously damped with a few drops of a saturated solution of ammonium sulphate. The filtrate should be transferred back to the precipitation tube and after mixing well, filtered again. This process prevents loss of any bile salts which may have escaped through the filter paper with the first few drops of the filtrate. The filtrate can now be discarded. The filter paper and precipitation tube are then washed with about 50 to 100 c.c. of saturated solution of ammonium sulphate, transferring about 20 c.c. of this at a time to the precipitation tube and after stoppering and shaking vigorously, poured on to the filter paper. In this manner traces of bile salts adhering to the boiling tube and the stopper are removed as well as the HCl introduced in the first stage of the process. Most

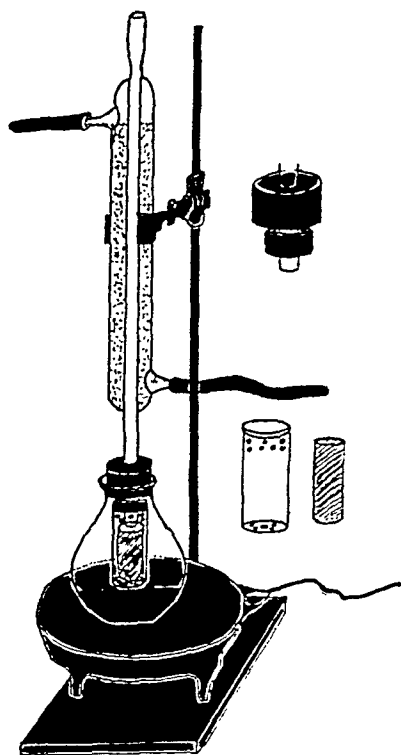


Fig. 2.

of the interfering urinary pigments are also removed by this washing. The washed paper and funnel are allowed to dry overnight in the incubator at 37° C. It has been found possible to dry the filter paper containing the bile salts in a hot air oven at about 90° C. to 100° C. for one hour. The ordinary copper still oven is superior to a flame heated oven and more convenient. The dried paper containing the bile acids is carefully folded, or better still cut into small strips, and inserted into a paper extraction thimble (Whatman thimbles, 22 by 80 mm.). The salts are then subjected to steady extraction with about 25 c.c. of absolute alcohol for sixty minutes, using the modified Soxhlet extraction apparatus (see Fig. 2) recommended by Myers^s for the estimation of cholesterol in blood. An electric hot plate is used for heating and

great care must be taken not to char the solution by excessive heat. The alcoholic extract is then removed, cooled, and transferred to a 50 c.c. measuring flask, rinsing out the extraction flask with small quantities of absolute alcohol and transfer the washings to the graduated flask, until the volume reaches about 35 to 40 c.c. An alternative but not so reliable method is to place the filter paper containing the bile acids into one of the large boiling tubes and extract with about 5 c.c. portions of absolute alcohol brought to boiling point each time by immersion of the tube in the boiling water-bath. The boiling of the alcohol should be continued for two or three minutes after ebullition. The alcoholic extract is then cooled and filtered through a small 7 cm. paper into the 50 c.c. flask to remove traces of ammonium sulphate. Extraction can be discontinued when 30 to 35 c.c. of alcohol have been used. Two cubic centimeters of a saturated solution of barium hydroxide is then added, and after the addition of a few porous plate chips to prevent bumping, the flask is placed in the boiling water-bath, and the alcohol gently boiled for about three minutes when the total volume will be reduced by about one-tenth. The flask is then removed and thoroughly cooled in running water after which the volume is made up to the mark with absolute alcohol. It might be mentioned that this barium hydroxide treatment does not remove any bile salts from the alcoholic extract. Quantitative figures show an absolute recovery of bile salts.

It is essential to cool the alcoholic extract thoroughly after boiling with the barium hydroxide. If convenient the solution should be allowed to stand from one to two hours so as to allow the baryta treatment to remove the last traces of urinary pigment. A quicker process is to stand the flask in the ice chest for about one hour, or until the temperature of the mixture is round about 5° C. A convenient freezing mixture is ice and dilute sulphuric acid which gives a temperature of about -10° C. (Approximately 50 gm. ice and 60 c.c. of 25 per cent H_2SO_4 .) Another good mixture is made as follows: 10 gm. of ammonium nitrate, 10 gm. of sodium carbonate, and 10 c.c. of water. The temperature reached is about 0° C.

After mixing well, filter through a 9 cm. paper that has been previously dampened with a few drops of alcohol. Slight turbidity of the filtrate can be safely overlooked as this does not interfere with the subsequent determination.

Forty-five cubic centimeters of filtrate are measured into one of the boiling tubes, and after the addition of a few silica chips, the alcohol is evaporated to dryness on the boiling water-bath. If the boiling tube is unable to hold 45 c.c. filtrate, first evaporate 25 c.c. of extract almost to dryness and then add the other 20 c.c. of filtrate together with another silica chip to prevent bumping. Keep the water temperature of the bath from about 85° to 90° C. at the beginning of the evaporation and raise the temperature to boiling as the alcoholic solution slowly reduces in volume.

It may occasionally be found that owing to the presence of water which was introduced with the saturated barium hydroxide solution, the last traces of alcohol cannot be removed. The addition of a further 10 c.c. of alcohol will make it possible to evaporate the extract to complete dryness.

It is usual to employ two standards for the urinary process, that is, 0.20 and 0.50 c.c. of the 0.5 per cent alcoholic solution of sodium tauroglycocholate. The alcohol is removed by evaporation on the boiling water-bath.

The color production is now performed on the dried residues. One cubic centimeter of 1 per cent levulose is added to each of the standards and to the unknown residue, and after agitating each tube to break up the precipitate and facilitate solution, 9 c.c. of pure concentrated A.R. hydrochloric acid is then added to all three tubes. After mixing thoroughly, the tubes are securely stoppered and placed in a water-bath for thirty minutes at 38° C. Occasional agitation of the tubes is recommended to ensure an even emulsion and production of color. If bile salts are present a reddish purple color slowly develops which is characterized by a beautiful greenish blue fluorescence. After thirty minutes when the colors have reached a maximum depth, the tubes are removed from the water-bath and cooled in running water. The unknown solution is filtered through a 9 cm. paper, and if the standards are cloudy (and this is always experienced with the stronger standards) they also must be filtered. Select the standard that is nearest the unknown solution by naked eye observation, and then compare in the colorimeter immediately, as fading occurs on standing. As a supplement to the method introduced above, a technic based upon the color production from phosphoric acid and levulose has been utilized. Exactly the same technic of color production is adapted as in the former method, namely, 1 c.c. of 1 per cent levulose and 9 c.c. of pure concentrated A.R. phosphoric acid, but heating is continued for one hour at 38° C. This latter technic, although not so delicate as the hydrochloric acid method somewhat compensates for this drawback, in that the color production is quite stable and also develops to double or even treble the intensity of the original color on standing twenty-four hours. The precipitate in the unknown solution is slightly more difficult to remove with the phosphoric acid method and requires filtration through an asbestos mat in a Gooch crucible (see Fig. 1). A test tube is placed in the pressure flask to collect the filtrate. This may have to be refiltered before it is absolutely clear for colorimetric comparison. The hydrochloric acid treatment will, however, be found to be the more convenient for rapidity of strong color production.

For the experimental work portions of the alcoholic sodium tauroglycocholate of known concentration were measured into the boiling tubes and evaporated to dryness on the boiling water-bath. After the addition of heavily pigmented urines, rich in chromogens and uroerythrin, pure bilirubin was also added in large quantities. The urines were then subjected to the method given above. The results are recorded in Table I and shows the method to be strictly quantitative.

In cases when the estimation of bile salts in test meal (duodenal fluids) samples is necessary, or for the estimation of bile salts in gallbladder bile, the urine technic can be applied after suitable dilution of the specimens, although a direct method of extraction with alcohol is quicker.

Lyon⁹ has claimed that it is possible to obtain fractions after the administration of magnesium sulphate which corresponds to the bile, A from the bile

TABLE I

OBSERVED COLORIMETRIC FINDINGS OF STANDARD SOLUTION OF VARYING CONCENTRATIONS OF BILE ACID ADDED TO URINE, AND ALSO THE RECOVERY OF THE NATURAL BILE ACIDS FROM ICTERIC URINES

SERIES	OBSERVATIONS ON URINE EXAMINED	HAY'S TEST	TYPE OF REACTION	SOD. TAURO-GLYCO-CHOLATE ADDED IN MG.	RECOVERED VALUES IN MG.	CORRECTION FOR MG. PER CENT	
						ADDED	RECOVERED
1	Pure solid bilirubin added to the extent of 20 units on V. den Bergh's scale	--	--	20.0	19.0	80.0	76.0
2	Urine contained heavy pigments, with pure bilirubin added to extent of 25 units	--	--	1.25	1.20	5.0	4.8
3	Heavy pigmented urine, added bilirubin 10 units	--	--	1.0	0.80	4.0	3.2
4	Heavily pigmented urine	--	--	0.5	0.52	2.0	2.08
5	Urine rich in urinary pigments. Solid bilirubin added. Bile acids recovered by repeated washings with alcohol and not Soxhlet extraction	--	--	50.0	49.4	200.0	197.6
6	Same as above but using Soxhlet extraction process	--	--	50.0	50.0	200.0	200.0
7	Urine contained very heavy pigments and chromogens. Final filtrate slightly contaminated with pigments	--	--	50.0	54.0	200.0	216.0
8	Urine very heavy in pigments	--	--	10.0	8.6	40.0	34.4
9	Gallbladder bile. 1 in 50 dil. made of pure bile and 25 c.c. (0.45) taken for analysis	--	--	--	17.5	--	70.0 i.e. 3885.0
10	Direct extraction made from the pure bile used above. 0.55 c.c. taken into 50 c.c. alcohol and 40 c.c. extract finally used, viz.: 0.44 c.c.	--	--	--	18.0	--	72.0 i.e. 4086.0
11	Pure bile added to urine. Control made with water	--	--	5.0	5.0	20.0	20.0
12A	Bilirubin in urine—7 units. Natural bile salts present	Very faintly positive	Direct coloration in the cold on addition of color producing reagents, which faded slightly on heating at 35° C.	--	4.0	--	16.0
13	Urine contained large amount of bile pigment (15 units)	Very faint trace	Immediate color development which developed still more on heating	--	1.1	--	4.4

TABLE I—CONT'D

SERIES	OBSERVATIONS ON URINE EXAMINED	HAY'S TEST	TYPE OF RE-ACTION	SOD. TAURO-GLYCO-CHOLATE ADDED IN MG.	RECOVERED VALUES IN MG.	CORRECTION FOR MG. PER CENT	
						ADDED	RECOVERED
14	Urine contained fair amount of urobilinogen (9.3 units). No bile pigments. 50 c.c. urine used	Markedly positive.	Color developed immediately and faded very slightly on heating	--	2.5	--	5.0
15B	Bilirubin in urine 12 units	Very faint trace	No development of color in the cold, but gradual development of color on heating at 35° C. This is the same as for all body fluids other than urine, and also for the synthetic bile salts	--	2.4	--	9.5
16	Repeated as above but using the H ₂ PO ₄ technique	--	--	--	2.3	--	9.3
17	Urine contained large amount of uroerythrin	Trace present	No immediate color but gradual development on heating in water-bath	--	0.95	--	1.9
18	Repeated but with uroerythrin first removed	--	--	--	0.95	--	1.9
19	Urine contained bilirubin, 14 units. 50 c.c. used	Very faint trace	Immediate color in the cold which faded slightly on heating	--	0.80	--	1.6
20	Urine contained 10 units of bilirubin	Faint trace	Immediate color in the cold which entirely faded on heating	--	--	--	--
21	No urobilin or bile pigments. Urine had a slight icteric tint	Very strongly positive	Absolutely negative	--	--	--	--
22C	Large amount of bilirubin present in urine, 17 units	Trace present	Direct color with complete fading on incubation	--	--	--	--
23	Urine containing urobilinogen and urobilin with trace of uroerythrin and bilirubin	Trace present	Very heavy direct coloration in the cold with complete fading on heating	--	--	--	--
24	Urine very heavily pigmented but with only a trace of bile pigments present	Strongly positive	Negative	--	--	--	--
25	Urine heavily pigmented with large amount of bilirubin	Faint trace	No immediate color in cold but gradual development of color on heating	--	0.50	--	2.0
26D	Catarrhal jaundice. Urine contained 5 units of bilirubin. Trace of uroerythrin. 50 c.c. used	Strongly positive	No immediate color in cold but gradual development of color on incubation	--	9.3	--	18.6

TABLE I—CONT'D

SERIES	OBSERVATIONS ON URINE EXAMINED	HAY'S TEST	TYPE OF REACTION	SOD. TAUROGLYCOCHOLATE ADDED IN MG.	RECOVERED VALUES IN MG.	CORRECTION FOR MG. PER CENT	
						ADDED	RECOVERED
27	Test meal sample (bile stained). 10 c.c. used	Strongly positive	--	--	10.0	--	100.0
28	Urine very heavily pigmented containing moderate amount of bilirubin (11 units)	Strongly positive	No coloration in the cold but gradual development on heating	--	3.75	--	15.0
29	Same as above but using the H_2PO_4 method	--	--	--	3.83	--	15.3
30	Chronic interstitial nephritis. A second estimation was performed allowing the color to develop in the cold for 10 minutes and comparing colorimetrically. 50 c.c. of urine taken	Faintly positive	Strong immediate coloration in the cold with fading on heating at 35° C.	--	2.30	--	4.60
31	Heavily pigmented urine containing fair amount of urobilinogen and large amount of bilirubin, 12 units	Very strongly positive	No coloration in the cold, but strong development of color on incubation	--	5.3	--	21.2
32	Acute nephritis. Second estimation without incubation, but allowing the color to develop in the cold for ten minutes and comparing colorimetrically. 50 c.c. of urine used	Markedly positive	Very strong immediate coloration in the cold with fading on incubation	--	11.7	--	23.4
33	Carcinoma of liver. Urine contained no bile pigments but a large amount of uroerythrin. 50 c.c. of urine used	Faint trace	An immediate coloration in the cold with slight fading on incubation	--	2.0	--	4.0
34	Same as above but using the H_2PO_4 method	--	Immediate coloration in the cold but no further development on heating	--	2.4	--	4.8

ducts, B from the gallbladder, and C from the liver, but other workers do not agree that the bile from these three sources can be fractionated successfully. The most important part of the analysis is the bilirubin and bile salts fractions.

Chiray and Lebon state that with bilirubin and bile salts there may be little or nil in obstructive jaundice, but an increase in familial jaundice, chronic splenomegalic icterus, hemolytic jaundice, paroxysmal hemoglobinuria and pernicious anemia. In catarrhal jaundice the concentration of bilirubin and bile salts may be normal, but the total output low. There may be an increase or decrease of bile salts in parallel with bilirubin, or there may be "dissociation" as in hemolytic jaundice (see Chiray and Lebon's book, *Le Tubage Duodenal*, 1924).

In the above book Carnot and Mauban describe a technic employing Hay's test¹⁰ and have thereby obtained an approximate measurement. The new process is introduced to give a strictly quantitative method.

With test meal samples dilute 2 or 5 c.c., depending upon the icteric tint of the specimen, to 25 c.c. with water and proceed as for the urine process. For the direct extraction add the above amount of gastric juice into about 30 c.c. of alcohol, add a few porous chips and heat on the water-bath for two or three minutes. After cooling, dilute to 50 c.c. with alcohol. Filter through a 9 cm. paper. Forty-five cubic centimeters of filtrate are pipetted into a 100 c.c. capacity Erlenmeyer flask, 3 c.c. of the saturated solution of barium hydroxide are added plus a few porous chips, and the mixture is gently boiled on the boiling water-bath for about three minutes after which it is thoroughly cooled in running water. Now transfer it to a small measuring cylinder and make up the total volume of the mixture to 45 c.c. with alcoholic washings from the Erlenmeyer flask. Filter through a 9 cm. paper. Forty cubic centimeters of alcoholic filtrate are pipetted into a boiling tube, and after the addition of a few silica chips, evaporated to dryness on the water-bath. The color production is exactly the same as for the urinary process. The standard usually employed is 1 c.c. of the 0.5 per cent standard solution.

For Gallbladder Bile.—Twenty-five cubic centimeters of 1 in 100 dilution of gallbladder bile is treated exactly as for the urinary process. With the direct extraction 0.25 c.c. of pure bile is added by means of a blood pipette to about 30 c.c. of alcohol, after which the test meal method for the direct extraction of bile acids is followed. Two cubic centimeters of the 0.5 per cent solution is used as standard.

The strength of the color production is reported in terms of milligrams of tauroglycocholate for each 100 c.c. of urine, calculated by the formula:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \frac{\text{Strength of standard (1 or 2.5 mg.)}}{\text{Amount of urine used (25 c.c.)}} \\ \times \text{Fraction used } \left(\frac{50}{45} \right) \times 100 = \text{mg. of bile salts per 100 c.c. of urine.}$$

Interpretation of Results.—An interesting fact emerges from this reaction, namely, the difference in color reaction from urinary extracts may be due to two distinct types of bile salts, Types A and B.

With Type A, the reaction is the usual response always obtained from all other body fluids, viz., no production of color in the cold, but a gradual development of color reaching a maximum in thirty minutes on warming in the water-bath at 38° C.

With Type B, the production of color begins immediately or after a few minutes in the cold, depending upon the amount of bile salts present. This coloration either develops slowly or fades entirely on heating. It is suggested that the B type of bile salts are exceedingly unstable with the result that the color development fades rapidly on heating. When, however, there is a combination of these two types of bile salts it will be observed that the color pro-

duced in the cold appears to develop on heating. The explanation may be that the B type of bile salts is stabilized by the presence of the A type, or again, on heating, the color developed from the A type of bile salts entirely obscures the fading of the color from the B type.

To overcome this difficulty the color that develops in the cold should be allowed to stand a further fifteen minutes, and after filtration compared in the colorimeter against a suitable standard prepared by heating at 38° C. in the usual way. The other process can be used by which the color can be stabilized, namely, the utilization of the phosphoric acid technic. This method will have to be adopted for all specimens of urine, as, of course, it is impossible to know when a direct production will be obtained, or again, if this coloration will develop or fade on incubation. But as already stated the phosphoric acid technic is not so convenient as the hydrochloric acid treatment, in that with the majority of urines containing small amounts of bile salts, the specimens must be allowed to stand for twenty-four hours until sufficient color has developed for colorimetric comparison.

The new reaction also disagrees with Hay's test for bile salts. This is to be expected as Hay's test depends on a lowered surface tension of the urine, and therefore is not specific for bile acids. It has been observed that substances lowering the surface tension may occur in certain cases of nephritis. Urines examined from interstitial and acute nephritis and giving a positive Hay's test show the characteristic reaction of the B type of bile salts.

Therefore, it must be concluded, by the new reaction proving these so-called "substances" to be bile salts, that the nephritic toxemias cause derangement of hepatic function. Recapitulating, it may be said that the B type of bile salts do not react to give the characteristic hydrochloric acid levulose-colored compound, and also, the majority of these bile salts remain in the filtrate from the urine and are therefore not precipitated with hydrochloric acid and ammonium sulphate. It is quite possible that the latter part of the theory given below explains these interesting findings.

The difference in the color reaction may depend either upon some slight variation in the bile salt molecule, or upon bile salts being present in some sort of association with cholesterol or a urinary constituent, which might well modify their physical properties.

Another interesting fact is that the cholesterol in the gallbladder is kept in solution as an emulsoid colloid as a result of the solvent action of bile salts. With deranged hepatic function the close association of cholesterol and bile acids makes the above theory feasible.

These are the observed facts, and it is idle to speculate as to their meaning until more is known of the physiology of the subject.

For Blood.—Into a 50 c.c. measuring flask containing about 30 c.c. of absolute alcohol, add 5 c.c. of whole blood, serum, or plasma. Immerse the flask in the boiling water-bath with constant agitation for two or three minutes, cool, and dilute to the 50 c.c. mark with alcohol. The protein precipitate should be allowed to separate roughly before filtering as this saves considerable time with filtration. The supernatant fluid is then filtered through a 9 cm. paper.

Transfer 40 c.c. of filtrate to a 100 c.c. Erlenmeyer flask, add 1 c.c. of saturated solution of barium hydroxide, and after adding a few small porous chips place in the boiling water-bath for about two minutes. The mixture is thoroughly cooled, transferred to a measuring cylinder and the volume made up to 40 c.c. with alcoholic washings from the Erlenmeyer flask. After filtering through a 9 cm. paper, 35 c.c. filtrate are pipetted into a boiling tube, a few silica chips are added, and the alcoholic extract is taken to dryness on the boiling water-bath. If frothing takes place toward the end of the evaporation this can be prevented by the addition of one or two drops of caprylic alcohol. The standard is prepared by taking 5 c.c. of a 0.005 per cent solution of sodium tauroglycocholate (1 c.c. of 0.5 per cent solution diluted to 100 c.c. with absolute alcohol) to dryness on the water-bath.

The colors are produced in a volume of 2.5 c.c., i.e., 0.25 c.c. of 1 per cent levulose and 2.25 c.c. of strong concentrated hydrochloric acid. The "unknown" solution is too small to be filtered and must be transferred to a centrifuge tube, and after securely stoppering the tube, centrifuge at high speed. Although by centrifuging the supernatant fluid never becomes absolutely clear this does not annul the delicacy of the reddish purple coloration. The unknown solution is then compared against the standard color in a similar tube by naked eye observation. If the unknown color is stronger than the standard solution it must be diluted with pure concentrated A.R. hydrochloric acid until the colors match. On the other hand if the standard solution is the stronger color, it must be diluted likewise with the hydrochloric acid. The volumes of both the solutions are now taken. This process must be called simply "an estimation," and not a quantitative determination.

$$\frac{\text{Volume in centrifuge tube of unknown solution}}{\text{Volume in centrifuge tube of standard solution}} \times \frac{\text{Strength of standard in mg.}}{\text{Amount of blood used}}$$

$$\frac{50}{35} \times 100 \text{ equals mg. of bile acids per 100 c.c. of blood.}$$

There can be little doubt that a mechanism for the manufacture of bile salts exists in the living body. In the presence of a biliary fistula such salts are continually excreted which would be impossible but for their endogenous formation. Moreover, those bile salts excreted into the duodenum are re-absorbed lower down in the intestinal tract, for none can be detected in the feces. They must therefore be circulating in the portal circulation and unless picked up quantitatively by the liver must circulate in the general blood stream and should therefore be detectable. Although some of the workers quoted earlier were unable to demonstrate the presence of bile salts in normal blood, it is by no means justifiable to conclude that they are absent. The results recorded in Table II clearly show that small quantities of bile salts added to normal blood in vitro could not be recovered. In fact on the average 5 mg. of bile salts were always lost, whatever the quantity added to 100 c.c. of blood. Lesser quantities were completely lost. It is assumed that they were carried down with the protein precipitate on the addition of alcohol, though in what form it is difficult to say. It may be possible that the bile salts are in combination with one of the constituents of the protein precipitate in which form

TABLE II

SHOWING RECOVERY OF BILE ACIDS ADDED TO BLOOD AND FINDINGS IN VARIOUS CONDITIONS

SERIES	OBSERVATIONS ON BLOOD EXAMINED	SOD. TAURO-GLYCOCHOLATE ADDED IN MG.	RECOVERED VALUES IN MG.	CORRECTION FOR MG. PER CENT	
				ADDED	RECOVERED
1	0.20 mg. of bile salts added to 5 c.c. blood plasma. Color produced in volume of 5 c.c.	0.20	0	4.0	0
2	0.25 mg. of bile salts added to 5 c.c. serum. Color produced in 2.5 c.c. volume	0.25	0	5.0	0
3	0.25 mg. of bile salts added to 5 c.c. blood plasma. The alcoholic extract was taken direct to dryness and not subjected to the barium hydroxide treatment. Color produced in volume of 2.5 c.c.	0.25	0	5.0	0
4	0.25 mg. of bile salts added to 5 c.c. whole blood, color produced in volume of 2.5 c.c.	0.25	0	5.0	0
5	1 mg. of bile salts added to 5 c.c. of whole blood; color in 7 c.c.	1.00	0.75	20.0	15.0
6	0.5 mg. of bile salts added to 5 c.c. whole blood; color in 5 c.c.	0.50	0.30	10.0	6.0
7	5 mg. of bile salts added to 5 c.c. whole blood	5.0	4.5	100.0	90.0
8	7 c.c. of normal plasma taken for analysis	--	--	--	0
9	10 c.c. of normal serum used	--	--	--	0
10	10 c.c. whole blood	--	--	--	0
11	20 c.c. whole blood	--	--	--	0
12	10 c.c. whole blood treated as for the Aldrich and Bledsoe process	--	--	--	0
13	Blood giving delayed Van den Bergh reaction; indirect reaction 4.7 units. 5 c.c. of serum used	--	--	--	0
14	Pernicious anemia. Van den Bergh reaction, indirect only, giving 18 units	--	--	--	7.0
15	Splenic anemia. 4 c.c. blood taken, and color produced direct from alcoholic extract	--	--	--	0
16A	Blood gave indirect Van den Bergh reaction. 5 c.c. of whole blood used	--	--	--	9.0
17B	A strong immediate Van den Bergh reaction. 4 c.c. of serum used	--	--	--	23.0
18C	Very strong immediate direct Van den Bergh reaction. 50 units of bilirubin	--	--	--	47.0
19	Blood giving positive indirect Van den Bergh test of 5 units	--	--	--	0
20D	Catarrhal jaundice. Immediate direct reaction. 7 units of bilirubin present	--	--	--	0
21	Jaundice. Biphase Van den Bergh reaction. 11 units of bile	--	--	--	0

they actually circulate in the blood stream. Or again, the blood proteins may possess the property of "adsorbing" a certain quantity of the bile salts after which these cannot be redissolved by the absolute alcohol. This seems most likely as the ratio between bile salts and blood protein per 5 c.c. of blood is 0.25 and 350.0 mg. respectively. The amount of bile salts lost varies slightly with the total protein content of the blood.

Transfer 40 c.c. of filtrate to a 100 c.c. Erlenmeyer flask, add 1 c.c. of saturated solution of barium hydroxide, and after adding a few small porous chips place in the boiling water-bath for about two minutes. The mixture is thoroughly cooled, transferred to a measuring cylinder and the volume made up to 40 c.c. with alcoholic washings from the Erlenmeyer flask. After filtering through a 9 cm. paper, 35 c.c. filtrate are pipetted into a boiling tube, a few silica chips are added, and the alcoholic extract is taken to dryness on the boiling water-bath. If frothing takes place toward the end of the evaporation this can be prevented by the addition of one or two drops of caprylic alcohol. The standard is prepared by taking 5 c.c. of a 0.005 per cent solution of sodium tauroglycocholate (1 c.c. of 0.5 per cent solution diluted to 100 c.c. with absolute alcohol) to dryness on the water-bath.

The colors are produced in a volume of 2.5 c.c., i.e., 0.25 c.c. of 1 per cent levulose and 2.25 c.c. of strong concentrated hydrochloric acid. The "unknown" solution is too small to be filtered and must be transferred to a centrifuge tube, and after securely stoppering the tube, centrifuge at high speed. Although by centrifuging the supernatant fluid never becomes absolutely clear this does not annul the delicacy of the reddish purple coloration. The unknown solution is then compared against the standard color in a similar tube by naked eye observation. If the unknown color is stronger than the standard solution it must be diluted with pure concentrated A.R. hydrochloric acid until the colors match. On the other hand if the standard solution is the stronger color, it must be diluted likewise with the hydrochloric acid. The volumes of both the solutions are now taken. This process must be called simply "an estimation," and not a quantitative determination.

$$\frac{\text{Volume in centrifuge tube of unknown solution}}{\text{Volume in centrifuge tube of standard solution}} \times \frac{\text{Strength of standard in mg.}}{\text{Amount of blood used}} \\ = \frac{50}{35} \times 100 \text{ equals mg. of bile acids per 100 c.c. of blood.}$$

There can be little doubt that a mechanism for the manufacture of bile salts exists in the living body. In the presence of a biliary fistula such salts are continually excreted which would be impossible but for their endogenous formation. Moreover, those bile salts excreted into the duodenum are re-absorbed lower down in the intestinal tract, for none can be detected in the feces. They must therefore be circulating in the portal circulation and unless picked up quantitatively by the liver must circulate in the general blood stream and should therefore be detectable. Although some of the workers quoted earlier were unable to demonstrate the presence of bile salts in normal blood, it is by no means justifiable to conclude that they are absent. The results recorded in Table II clearly show that small quantities of bile salts added to normal blood in vitro could not be recovered. In fact on the average 5 mg. of bile salts were always lost, whatever the quantity added to 100 c.c. of blood. Lesser quantities were completely lost. It is assumed that they were carried down with the protein precipitate on the addition of alcohol, though in what form it is difficult to say. It may be possible that the bile salts are in combination with one of the constituents of the protein precipitate in which form

well. After adding a few porous chips immerse the tube in the boiling water-bath for two to three minutes, cool, and dilute to 50 c.c. with absolute alcohol. Allow the precipitate roughly to settle and then carefully pour off supernatant extract on to a 9 cm. paper. Transfer 40 to 45 c.c. of filtrate to a 100 c.c. capacity Erlenmeyer flask, add 2 c.c. of the saturated solution barium hydroxide plus a few porous chips. Heat on the boiling water-bath for about three minutes or until the mixture has concentrated about one-tenth. After thoroughly cooling the solution in running water, make up to the original volume (viz. 40 or 45 c.c.). Filter through a 9 cm. paper. Pipette 35 or 40 c.c. of filtrate into a boiling tube together with a few silica chips, and evaporate to dryness on the water-bath.

It will be noticed that the fecal residue contains much more pigment than either the urine or the blood extracts, but this does not annul the subsequent color production as the pigments and fats are rendered insoluble by the hydrochloric acid introduced in the color production stage of the method. The

TABLE III

SHOWING EFFECT OF BILE ACIDS ADDED TO FECES AND THEIR SUBSEQUENT RECOVERY

SERIES	OBSERVATIONS ON FECES EXAMINED	SOD. TAURO- GLYCO- CHOLATE ADDED IN MG.	RECOVERED VALUES IN MG.	CORRECTION FOR MG. PER CENT IN WET FECES		CORRECTED FOR DRY WEIGHT	
				THEORET- ICAL	FOUND	THEORET- ICAL	FOUND
1	Normal feces. Two grams wet feces used. Solid material 30.00 per cent	5.0	4.4	250.0	220.0	833.0	733.0
2	Normal feces. Three grams wet feces used. Solid percentage 14.30	5.0	4.7	166.6	156.6	1650.0	1095.0
3	Gross diarrhea. Three grams wet feces used. Solid percentage 8.33. Bilirubin present to extent of 93 units per 100 gm. of dry feces	--	2.1	--	70.0	--	840.0

insoluble fats and pigment are then removed by filtration. Two standards should be prepared 0.2 and 0.5 of the 0.5 per cent standard solution. The color production is the same as for the urinary process.

Calculation:

$$\frac{\text{Standard reading}}{\text{Unknown reading}} \times \frac{\text{Strength of standard in mg.}}{\text{Amount of feces taken}} \times \frac{50}{35} \text{ (or the amount of alcoholic extract finally taken to dryness)} \times 100 = \text{mg. of sodium tauroglycocholate per 100 gm. of wet feces.}$$

To obtain the solid percentage of the feces examined, weigh out exactly 1 gm. of wet feces into a small porcelain dish and dry by means of a boiling water-bath and vacuum desiccator. The weight of the remaining residue multiplied by 100 gives the solid material expressed in percentage. Now if the amount of bile salts present in 100 gm. of wet feces is multiplied by:

$$\frac{100}{\text{Solid percentage of feces}},$$
 the percentage of bile salts per 100 gm. of dried feces will be ascertained.

The present investigation proves definitely that the blood is able to conceal a small quantity of bile salts so that they escape detection. Those workers, therefore, who found bile salts absent in normal blood, actually found only their absence above a certain concentration, i.e., 5 mg. per 100 c.c. of blood. Whatever the amount of blood used the amount of bile salts carried down by the protein precipitate remains proportionally constant, viz., 5 mg. per 100 c.c. of blood. Furthermore, with methods employing a latter stage in the alcoholic extract, such as the addition of morite to remove coloring matter, a large quantity of bile salts are also removed. No matter what method is used to isolate the bile salts (and extraction with alcohol is by far the most successful), a small amount of bile acids is lost in the albuminous precipitate, which again varies considerably with the protein precipitant.

With a view to recovering the bile salts from the protein precipitates various methods were tried, such as, first the drying of blood on filter paper and extracting with absolute alcohol in the modified Soxhlet apparatus for one or two hours. Second, blood protein was removed by precipitation with absolute alcohol and then collected and subjected to steady extraction with absolute alcohol in the Soxhlet extraction apparatus from two to three hours. The results were negative in those samples to which bile salts had been added to the extent of 5 mg. per 100 c.c. of blood, as well as in bloods to which no bile salts were added.

Finally, investigation showed that even if the alcoholic extracts from very pale serums containing added bile salts (0.25 mg. per c.c. of blood) were taken direct to dryness and not subjected to the barium hydroxide treatment (there being practically no coloring matter to remove) absolute-negative results are obtained. In the above experiments the color production was performed in a total volume of 2.5 c.c., i.e., 0.25 c.c. of levulose and 2.25 c.c. of concentrated hydrochloric acid. The technic employed for the blood process is more useful as a qualitative than a quantitative method, first, because a reaction is only obtained when the amount of bile salts in the blood rises above 5 mg. per 100 c.c., and second, because a measurable color reaction cannot be obtained with quantities of blood less than 5 c.c. However, large quantities of blood can be used if available. An excess of bile acids can easily be demonstrated in the blood and the method is therefore very satisfactory.

With latent jaundice the test is rather unsatisfactory due presumably to the "holding back" of the bile acids in the protein precipitate. Here the estimation of bilirubin is superior. Further work, however, may give interesting results in connection with dissociated jaundice, i.e., independent retention or excretion of bile pigments and bile salts.

Hemolytic jaundice is certainly a dissociated condition, since the bile salts are unaffected. Retention of bile salts alone has been observed in the later stages of catarrhal jaundice.

For Feces.—Weigh out 2 gm. of wet feces with the aid of a glass rod, into one of the thick-walled boiling tubes that has been previously graduated at 50 c.c. If the feces is exceptionally liquid 3 gm. can be used. Add about 30 c.c. of alcohol and break up as much of the solid material as possible by stirring

containing 3 holes. Into one hole is fitted a thermometer; into the other 2 holes glass tubes are inserted. A piece of rubber tubing 15 to 18 cm. long is attached to one of the tubes on the inside surface of the stopper so that the circulation in the animal chamber produced by raising or lowering Bottle *A* will be efficient. Bottle *A* is filled with water and a small amount of copper sulphate introduced. If an antiseptic is not added to the water, the viscosity will be changed by bacterial growth, thus altering the emptying time of the bottle. The absorption bottles are filled two-thirds full with soda lime.* A suitable basin, either glass, galvanized iron or copper, measuring about 45 by 20 by 20 cm. is obtained and filled one-half full with water so that the animal chamber containing the guinea pig can be immersed when a determination is being made. This prevents sudden changes in the temperature of the air within the animal chamber. The walls of this basin should be painted black so that by placing cardboard or paper over the top, the animal is confined in a darkened chamber. This is an important factor in eliminating movement. The animal chamber should be anchored in the water basin and balanced in such a way that movement of the animal can be detected without admitting light to the water-bath.

The stoppers are fitted with glass tubing and the proper connections (see Fig. 1) made with a good grade of rubber tubing with fairly thick walls. The stoppers which need not be removed are sealed tightly with glue and all rubber to glass connection reinforced with wire. The rate of the flow of water from the "lift" Bottle *A* to Bottle *B* is regulated by the distance the "lift" Bottle is raised or lowered from the level of Bottle *B*. It should be regulated so that one bottle empties in exactly two or two and one-half minutes. Bottle *A* may be raised and lowered either by hand or by motor.

The system *must* be air tight. It is tested for leaks by inserting the stopper in the animal chamber, filling the spirometer with air and placing a 100 gm. weight on top of the spirometer. With Bottle *B* empty, the volume of gas in the spirometer is noted and Bottle *A* raised. When empty, it is lowered, and after Bottle *B* is again empty, the volume of gas in the spirometer is noted. If the volume of gas in the spirometer is the same as before the test, there should be no leaks, providing the temperature within the system is unchanged. Until one is convinced that the apparatus does not leak, a further test for leaks should be made after each metabolic determination by placing a weight on the spirometer with the water level in Bottles *A* and *B* stationary. The loss in volume within the spirometer should be no greater than the consumption of oxygen by the animal.

Method of Determination of Metabolic Test.—After immersing the animal chamber containing the guinea pig in the water-bath, the spirometer is filled with oxygen and the gas circulated for four or five minutes by raising and lowering Bottle *A*. After Bottle *B* is completely empty, the menisci in the inner and outer cylinders of the spirometer are adjusted to the same level and the volume of gas in the graduated cylinder, and the time noted. The temperature in

*We use Wilson's soda lime which is so extensively and satisfactorily used in determining the metabolic rate in human beings.

A SIMPLE AND INEXPENSIVE APPARATUS FOR THE DETERMINATION OF THE METABOLIC RATE IN GUINEA PIGS*

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WE WISH to present the details of an apparatus for the determination of the basal metabolic rate in guinea pigs, not because of any originality connected with its construction, but because of its simplicity and inexpensiveness. Several different types of apparatus were tried, but it required several weeks of experimentation with each to learn that, at least in our experience, the results became more constant as we simplified the apparatus. For animals as small as guinea pigs, we feel quite certain that the "closed system" type of apparatus is more practical than any other. All of the articles of glassware, etc., used in the construction are so simple that they can be found or constructed in almost any laboratory.

Description of Apparatus.—Two large glass bottles with a capacity of 2,300 c.c. to 2,500 c.c., like those used for containers of sulphuric acid, are fitted with rubber stoppers, and a hole about 2 cm. in diameter is bored in the bottom of each (see Fig. 1). One of these bottles (*B*) is fixed to a tripod on a table and the other (*A*) suspended with a cord over a pulley so that it can be lowered or raised. A thermometer is inserted through a hole in the stopper of the stationary bottle (*B*). Two bottles of about 500 c.c. capacity (to be used as absorption bottles) having large openings at the top are fitted with rubber stoppers, and a glass tube inserted through one of the holes in the stopper down to the bottom of each bottle. A small cage of wire screen is constructed and placed in the bottom of each bottle so as to prevent the hole of the tube from blocking with soda lime. A hole about 2 cm. in diameter is bored in the bottom of a 500 c.c. graduated cylinder and by means of a small pulley the cylinder is suspended into a larger glass cylinder measuring 45 cm. in height and 7 cm. in diameter. This serves as a spirometer. This latter cylinder is filled nearly to the top with water. A piece of glass tubing is bent in a narrow U-shaped fashion and anchored on the rim of the outer cylinder of the spirometer. The arm of the U-tube which is placed within the graduate cylinder is made long enough to protrude beyond the water level. The current of gas passing through this tube and out the top of the spirometer or vice versa, insures adequate circulation of the gas within the spirometer. A three-way glass stopcock is inserted between the spirometer and Bottle *B* so that oxygen may be introduced into the spirometer at the beginning of the metabolic test. A bottle with a capacity of about 1,500 c.c. and having a large hole in the top is used as the animal chamber (*F*). This jar is fitted with a rubber stopper

*From the Department of Surgery, Washington University School of Medicine and Barnes Hospital.

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containing 3 holes. Into one hole is fitted a thermometer; into the other 2 holes glass tubes are inserted. A piece of rubber tubing 15 to 18 cm. long is attached to one of the tubes on the inside surface of the stopper so that the circulation in the animal chamber produced by raising or lowering Bottle A will be efficient. Bottle A is filled with water and a small amount of copper sulphate introduced. If an antiseptic is not added to the water, the viscosity will be changed by bacterial growth, thus altering the emptying time of the bottle. The absorption bottles are filled two-thirds full with soda lime.* A suitable basin, either glass, galvanized iron or copper, measuring about 45 by 20 by 20 cm. is obtained and filled one-half full with water so that the animal chamber containing the guinea pig can be immersed when a determination is being made. This prevents sudden changes in the temperature of the air within the animal chamber. The walls of this basin should be painted black so that by placing cardboard or paper over the top, the animal is confined in a darkened chamber. This is an important factor in eliminating movement. The animal chamber should be anchored in the water basin and balanced in such a way that movement of the animal can be detected without admitting light to the water-bath.

The stoppers are fitted with glass tubing and the proper connections (see Fig. 1) made with a good grade of rubber tubing with fairly thick walls. The stoppers which need not be removed are sealed tightly with glue and all rubber to glass connection reinforced with wire. The rate of the flow of water from the "lift" Bottle A to Bottle B is regulated by the distance the "lift" Bottle is raised or lowered from the level of Bottle B. It should be regulated so that one bottle empties in exactly two or two and one-half minutes. Bottle A may be raised and lowered either by hand or by motor.

The system *must* be air tight. It is tested for leaks by inserting the stopper in the animal chamber, filling the spirometer with air and placing a 100 gm. weight on top of the spirometer. With Bottle B empty, the volume of gas in the spirometer is noted and Bottle A raised. When empty, it is lowered, and after Bottle B is again empty, the volume of gas in the spirometer is noted. If the volume of gas in the spirometer is the same as before the test, there should be no leaks, providing the temperature within the system is unchanged. Until one is convinced that the apparatus does not leak, a further test for leaks should be made after each metabolic determination by placing a weight on the spirometer with the water level in Bottles A and B stationary. The loss in volume within the spirometer should be no greater than the consumption of oxygen by the animal.

Method of Determination of Metabolic Test.—After immersing the animal chamber containing the guinea pig in the water-bath, the spirometer is filled with oxygen and the gas circulated for four or five minutes by raising and lowering Bottle A. After Bottle B is completely empty, the menisci in the inner and outer cylinders of the spirometer are adjusted to the same level and the volume of gas in the graduated cylinder, and the time noted. The temperature in

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Bottle *B* and the animal chamber is recorded. Bottle *A* is immediately lowered. The distance Bottle *A* is lowered or raised is adjusted so that the bottle will empty and fill in exactly five minutes. A record of the amount of oxygen consumed is thereby obtained every five minutes. It is necessary to continue the determination for at least twenty minutes so that any errors in reading gas volume, temperature, etc., are minimized. It is very important that gas

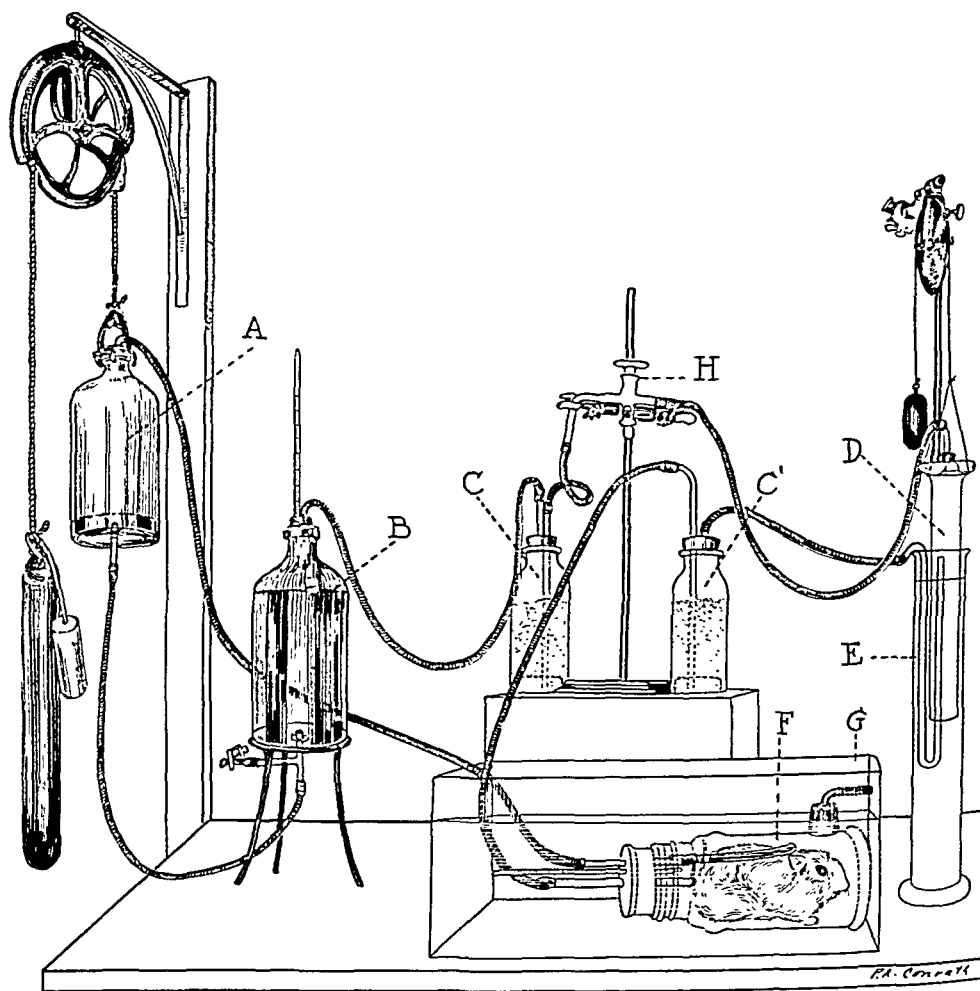


Fig. 1.—*A*, "Lift" bottle. *B*, Stationary bottle. *C*, *C'*, Absorption bottles containing soda lime. *D*, Graduated inner cylinder of spirometer. *E*, Outer cylinder of spirometer. *F*, Animal chamber. *G*, Water-bath. *H*, Three-way stopcock.

volume in the spirometer be recorded only when Bottle *B* is empty. If water is flowing from Bottle *A* to *B* or vice versa, the pressure required to move the water is sufficient to change the volume of gas in the spirometer. By recording the amount of oxygen consumption every five minutes, it is possible to detect errors which are brought about by movement, change in temperature, leaks, etc. At the end of the determination the temperature in the animal chamber and Bottle *B* is again recorded.

Before the volume of gas is reduced to 0° C. and 760 mm. pressure, adjustment must be made for any change in temperature within the system while the determination was being made. To make this correction, it is necessary to know the volume of the various components of the system. For every degree variation in temperature, an addition or subtraction of 0.004 c.c. for each cubic centimeter of gas in the system must be made, depending on whether the temperature rises or falls. It is probably more accurate to calibrate the system. To do this all draughts are eliminated from the room, and the temperature of the air within the bottles of the apparatus as well as the room must have been constant for at least thirty minutes before the calibration is made. The temperature of the water-bath containing the animal chamber is then raised 1° or 2° by the addition of hot water to the water-bath. To be accurate, an object approximately the size of a guinea pig, should be placed in the animal chamber before this calibration is made. The change in volume of air within the system for each degree change of temperature within the animal chamber is easily determined. In our apparatus 1° rise in temperature within the animal chamber produced an increase of 5.9 c.c. in the volume of the system as recorded by the spirometer. With the temperature of the animal chamber constant, a current of heated air is turned on Bottles A and B until the temperature has reached a constant temperature. With our apparatus a rise of 1° in the temperature of Bottle B caused an increase of 13.6 c.c. in the volume of the system as recorded by the spirometer.

Sample determination: Wt. of guinea pig 450 gm. Bar. Pr. 736 mm. Hours' starvation eighteen.

SPIROMETER READING	TIME		TEMPERATURE	
236	11:50	ANIMAL CHAMBER Bottle <i>B</i>	BEFORE	AFTER
206	11:55		27.6°	27.3° (drop of 0.3°)
176	12:00		27.3°	27.2° (drop of 0.1°)
145	12:05			
114	12:10			
<hr/> 122 c.c.				

Oxygen consumption in 20 minutes = 122 c.c.

Correction for drop in temperature in animal chamber $- 0.3 \times 5.9 = 1.77$ c.c.

Correction for drop in temperature in Bottle B. $0.1 \times 13.6 = 1.36$ c.c.

Total 3.13 c.c.

Oxygen consumption in 20 minutes (corrected) = $122 - 3 = 119$ c.c.

Oxygen consumption in 1 hour = 357; at 0° and 760 mm. = 304.5 c.c.

Oxygen consumption per kilo per hour = $\frac{304.5}{450} = 676$ c.c.

As long as the weight of the animal remains approximately the same, we find it more convenient and just as accurate to record the oxygen consumption in cubic centimeters per kilo per hour, instead of cubic centimeters or calories per square meter per hour. We feel that this method is just as accurate, especially when one considers that the shape of individual guinea pigs does not vary as it does in human beings. We do find it more accurate, however, to establish an average metabolic rate for each animal by making determinations every other day for several days, and to use that average as the metabolic

rate for that particular animal, since it is true that the metabolic rate of one animal may vary 10 or 12 per cent (see Table I) from that of another.

We have listed below a series of 10 animals upon which determinations were performed during the summer months and another series of 10 with determinations made during winter months. The figures represent an average of from 4 to 8 determinations upon that particular animal. The animals listed below represent consecutive experiments, except that out of each series of 10, one animal was discarded because of exceptionally high rate caused by uncontrollable motion of the animal.

TABLE I

METABOLIC RATE c.c. per kilo per hour	
10 ANIMALS IN SUMMER	10 ANIMALS IN WINTER
640	706
675	714
663	702
635	715
634	635
666	640
600	626
581	610
621	758
635	714
Average 635 c.c.	Average 682 c.c.
Variation of -6 to +8 per cent from the average	Variation of -9.5 to +11 per cent from the average

From the figures in the above table it will be observed that the average metabolic rate was approximately 7 per cent higher in winter than in summer. The individual variation was slightly greater in winter than in summer.

No attempt was made to weigh the various foods given the animals, but it is important that the diet is not deficient in certain necessary constituents. In our experience, feeding lettuce, carrots, oats, and alfalfa, would maintain a healthy normal guinea pig.

It is essential that animals of relatively the same age and weight be used. We have had the most consistent results with guinea pigs weighing between 400 and 480 gm. Any sudden loss of weight due to starvation or illness will lead to erroneous results (elevation of the metabolic rate).

We have made many determinations using a preliminary subcutaneous injection of 7 or 8 mg. sodium amytal twenty minutes before the test, and as far as we could determine obtained accurate results. The average oxygen consumption in consecutive animals receiving sodium amytal was 608 c.c. per kilo per hour, or 12 per cent lower than a series conducted at the same time without amytal. The individual variation in metabolic rate was -5 per cent and +8.5 per cent from the average, or very slightly less than the variation in animals not receiving amytal. The dose of 7 or 8 mg. for an animal of about 450 gm. was sufficient to make it close its eyes and lower its head as if asleep, but not enough to make it lie down. It recovers from the effects of this dose very rapidly but not before the determination is completed.

PRECAUTIONS

1. The system *must* be absolutely air tight. It should be tested frequently, if not after each determination by putting a weight on top of the spirometer and observing the gas volume within the cylinder.

2. At first thought, motion of the animals would appear to be a serious source of error. With the exception of an occasional animal, which must be discarded, this error can be eliminated, even though a guinea pig cannot be trained as can dogs or some other animals. Usually, darkening the chamber while the test is being made is of great advantage. If this is done, the animal chamber must be balanced in such a way so that motion can be detected. By recording the rate of consumption every five minutes, however, any movement will be quickly detected by the increased oxygen consumption. The animals must be starved at least fourteen hours, before each determination, but if starved for more than eighteen, they are apt to be restless on account of hunger. Guinea pigs do not tolerate cold quarters. If a metabolic determination is made while the animal is cold, it will, as expected, be elevated 15 to 25 per cent. It is therefore essential that the temperature of their quarters as well as the room where the determinations are made, be kept as warm as 24° C. and that the animal chamber also be kept warm (preferably 25° to 28° C.) while tests are being made. The apparatus must be protected from draughts of air. Although one can correct for changes in temperature within the system, this change will lead to less error if it is reduced to a minimum.

3. When gas volumes are recorded, the spirometer must be adjusted so that the two water menisci are on the same plane, and the reading must be made a few seconds after Bottle *B* has emptied.

4. Before each determination, the spirometer must be emptied and filled with oxygen, not air. If air is used, the depletion of oxygen within the system may lead to an enormous error (decrease in consumption as measured by the spirometer).

SUMMARY

We have described a simple apparatus, easily and cheaply constructed, which in our hands has yielded constant and presumably accurate results in determining the metabolic rate in small animals. We have used it only on guinea pigs, but see no reason why it could not be used for rats, especially if the sizes of the various chambers were reduced. By using proper precautions and discarding an occasional animal, movement can be entirely eliminated as a serious source of error. No claims for originality in construction of the apparatus are made.

A COMPARISON OF THE WASSERMANN AND KAHN REACTIONS UPON SPINAL FLUID IN TREATED AND UNTREATED CASES OF PARESIS*

DE LESTER SACKETT, B.S., AND ERIC ESELIUS, B.S., ELGIN, ILL.

A LARGE number of articles have appeared in the literature comparing the results of the Wassermann and Kahn reactions since Kahn introduced his test in 1922.¹ The Kahn reaction has been compared with many Wassermann technics, and at the present time it is generally accepted that there is a very close agreement between the results of the two tests upon blood serum.

Although we cannot agree with those individuals who, for various reasons, recommend that the Kahn can completely replace the Wassermann reaction upon blood serum as a routine procedure, we do believe that the Wassermann tests should likewise not be run independently. It is our opinion that the greatest value of any precipitation reaction is that it serves as a necessary check to the complement fixation reactions. All complement fixation reactions should be controlled by a recognized precipitation test, and vice versa, and never by a technic based upon the same underlying principles.

The Kahn reaction upon spinal fluid is also of value in the serology of syphilis, but has not been accorded the enthusiastic approval received by the same test upon the blood serum. Although many articles have been published containing a series of parallel Wassermann and Kahn reactions, which show a close agreement between the two tests, articles have appeared indicating that negative Kahn tests are frequently found upon spinal fluids of incompletely treated cases of neurosyphilis. This is in direct contrast to that found in the results of the reaction upon blood serum, for in this case it has been repeatedly pointed out that the Kahn reaction appeared to be slightly more sensitive than the Wassermann.

LITERATURE

Kahn² reported the comparative findings of the Wassermann and Kahn tests upon 750 spinal fluids as follows: perfect agreement, 670 (394 positive, 276 negative) results stronger in Wassermann, 44, results stronger in Kahn, 26, and in 10 anticomplementary Wassermanns, satisfactory results were obtained with the Kahn.

Again Kahn³ reported the findings of his test and the Harrison Wassermann method as follows: Four-plus reactions in the Wassermann 177, Kahn 223; 63 two-plus Wassermanns and 18 one-plus Kahns. Neither reaction gave any false positive results. These findings would indicate that the Kahn test was superior to this Wassermann technic.

*From the State Psychopathic Institute.
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Lederer¹ compared the results of the two tests upon 57 spinal fluids and found that the tests agreed upon 33 negative and 19 positives. There was one relative agreement and in four treated cases the Wassermann was more or less positive while the Kahn was negative. Using a modified Kahn technic he compared the two reactions upon 73 spinal fluids with the following results: 39 negative with both tests and 17 positives in perfect agreement, one fluid giving a weakly positive Wassermann gave a negative Kahn, and in 7 fluids the Wassermann was negative and the Kahn more or less positive.

Rabinovitch and Roberts² reported a series of 112 spinal fluids in which there was perfect agreement in 101 (63 negatives and 38 positives), and divergent results in 11 (4 stronger in the Wassermann, 6 in the Kahn), and one positive Kahn upon a fluid which gave an anticomplementary Wassermann reaction. They also observed that there was not any relation between the strength of the Kahn test and the amount of globulin present in the fluid.

Davenport³ examined 487 spinal fluids with the Kahn tests and obtained negative reactions upon 5 cases of neurosyphilis in a group of 118 diagnosed cases. He also obtained negative reactions upon 23 incompletely treated cases. The results of the Kahn test upon the remaining fluids in his group were in agreement with the clinical findings.

Hull, Fry and Garwood⁴ in 180 syphilitic cases found 135 positive and 45 negative Kahn reactions upon the spinal fluid. All but 14 of the negative tests were from patients who had received antisyphilitic treatment. On 229 fluids from patients classified as nonsyphilitic, 7 positive Kahn tests were procured. Of these 7 positive tests, 2 four-plus and 1 three-plus reactions were confirmed by positive gold sol reactions. The remaining had weak gold curves and weaker Kahn reactions. These authors conclude that the Kahn tests upon spinal fluid have a high degree of specificity in eliminating the possibility of cerebrospinal syphilis.

Germat and Serafimov⁵ compared the Kahn and Wassermann reactions upon the fluids of 50 cases with clinical syphilis. They found complete agreement between the two tests in 94.5 per cent and divergence in 5.5 per cent. In the latter the Kahn gave more positive reactions. In progressive paralysis treated with malaria and sulphur in oil, the Kahn retained a stability of the positive reaction. Only one case with prominent improvement of the patient gave a negative Kahn test. They conclude that the Kahn reactions have a fairly simple technic and deserve great attention.

This brief review of the literature does not pretend to cover the subject, but is offered to demonstrate the diversified opinion as to the value of the two reactions.

TECHNIC

Because of the great dilution of the spinal fluid, it is first necessary to concentrate the reacting substances in the fluid by the precipitation of the globulin with ammonium sulphate and then testing the redissolved globulin by the Kahn reaction.¹ Since ammonium sulphate in sufficient concentration is capable of producing a precipitate with the antigen, more salt solution must

be used in the antigen dilution. This is usually between 0.3 to 0.4 c.c. more per 1 c.c. of antigen than the standard titer.

It is evident that this weakening of the antigen would more or less affect the strength of the reaction obtained with its use. Kurtz and Larkum⁹ following the suggestion of Kahn and McDermott¹⁰ have devised a technic which sufficiently removes the excess ammonium sulphate so that the regular serum titer can be used. After precipitating the globulin with the sulphate and centrifuging the specimen to throw down the precipitate, they pour off the supernatant fluid and invert the tube in saline, dipping it up and down, taking care that the saline does not touch the precipitate at the bottom of the tube. The air is allowed to escape by means of a glass tube reaching from the entrapped air in the tube through the saline into the open air of the room. This procedure is carried out in triplicate on each specimen and the globulin dissolved in 0.3, 0.2, and 0.1 c.c. saline. They found stronger reactions in those tubes containing the more concentrated globulin solutions.

We have been able to accomplish the removal of the excess ammonium sulphate by a technic which we feel to be simpler and to have less opportunity for mechanical accidents. In using the technic of Kurtz and Larkum unless great care is used in rinsing the tube in the saline solution, there is a possibility of losing some of the globulin because of splashing. Globulin is very soluble in saline, so, of course, any saline coming in contact with it would result in a loss of globulin and reacting substances.

MODIFICATION OF TECHNIC

Our method differs from the technic just outlined in that after the globulin has been thrown down by centrifuging, the supernatant fluid is carefully pipetted off, the tube is placed in an inverted position upon some filter paper and allowed to drain for one-half hour. Then the inner sides of the tube are carefully dried by means of a cotton applicator, taking great care not to disturb the precipitated globulin. The globulin is then ready to be redissolved and the regular Kahn technic continued, except that the antigen serum titer can be used. In our study we did not concentrate the globulin dilution, but followed the routine Kahn procedure for spinal fluid.

In removing the supernatant fluid it was found that if great care was used, it could be poured instead of pipetted off without affecting the end-result of the test.

EXPERIMENTAL STUDY

We have compared the results obtained upon 202 spinal fluids with the Wassermann (Craig technic¹¹), standard Kahn, and modified Kahn (employing the serum antigen titer) reactions. This group consisted of 64 cases of untreated paresis, 90 cases of treated paresis, and 48 cases that were not considered to be paretics. The therapy used in the treated cases consisted of various fever therapies (diathermy, electric blanket, malaria and sulphur in oil) and tryparsamide. The spinal fluids were examined at a period ranging from two weeks following treatment to two years.

Considering the group as a whole there was a perfect agreement between

the three tests in 119 cases or 58.91 per cent (72 positives and 47 negatives). It would be very difficult to determine the relative agreement between the three tests, as many fluids had a relative agreement with two of the reactions but an absolute disagreement with the third test.

Chart 1 plots the curve of the perfect agreement of the spinal fluid Was-

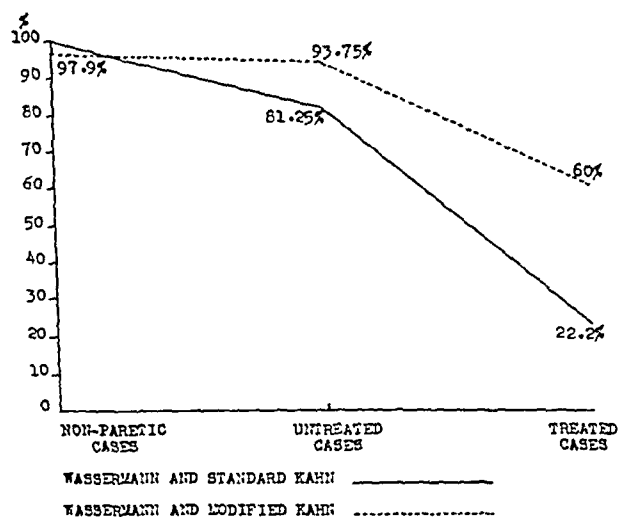


Chart 1.—Perfect agreement between spinal fluid Wassermann and Kahn reactions.

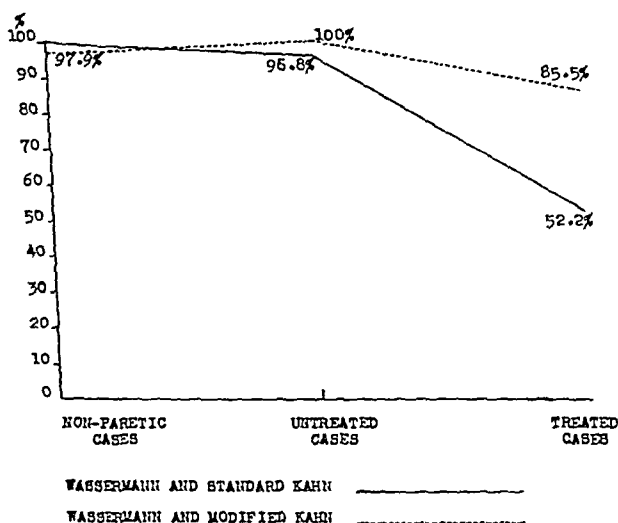


Chart 2.—Perfect or partial agreement between spinal fluid Wassermann and Kahn reactions.

sermann and the two Kahn reactions. There was 100 per cent agreement between the Wassermann and the standard Kahn in the 48 cases not diagnosed as paretics and having negative blood findings. In the 64 untreated cases of paresis, the agreement drops to 81.25 per cent or in 52 cases. In the treated cases this drops still further, or in only 20 of the 90 cases, or 22.2 per cent. There was one false two-plus reaction by the modified Kahn test among

the 48 nonparetics, thus making a 97.91 per cent agreement between this and the Wassermann reaction. In the untreated cases there were 60 cases in which there was a perfect agreement between the two tests or 93.75 per cent. This percentage dropped to 60 per cent or a perfect agreement in only 51 of the treated cases.

Chart 2 considers the sum of the perfect and relative agreement between the tests. The Wassermann and the Standard Kahn reactions had 100 per cent agreement in the nonparetics; in the untreated cases, 62 cases or 96.87 per cent had either a perfect or partial agreement, and in the treated cases this dropped to 52.2 per cent or 47 cases.

The Wassermann and modified Kahn reaction had a perfect or relative agreement in 47 of the nonparetic or 97.91 per cent, in the untreated cases

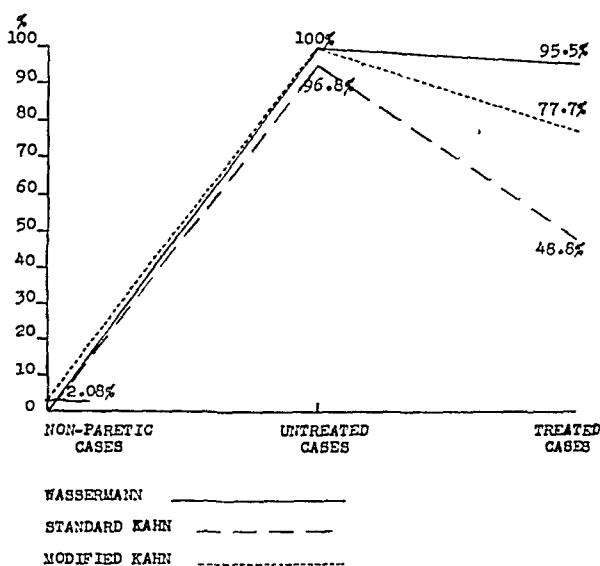


Chart 3.—Positive reactions in spinal fluid.

there was 100 per cent perfect or relative agreement and in the treated cases this agreement held true in 77 cases or 85.5 per cent.

Chart 3 plots the positive reactions of the three tests. There were no positive Wassermans in the fluids of the nonparetics, 100 per cent of the untreated cases of paresis had positive spinal fluid Wassermans and 86 cases or 95.5 per cent of the treated cases also had positive reactions with this test.

The standard Kahn reaction gave no positive reactions in the nonparetics; in the untreated cases, 62 or 96.87 per cent and in the treated cases, 44 or 48.8 per cent had positive reactions.

The modified Kahn test gave one false positive reaction in the nonparetic cases or 2.08 per cent, 100 per cent positive reactions in the untreated cases and in the treated cases 70 or 77.7 per cent had positive reactions.

Chart 4 demonstrates the comparative strength of the three reactions. The Wassermann and the standard Kahn both had all negative reactions in the nonparetics, but in the untreated cases the Wassermann was stronger than

the standard Kahn in 8 cases or 12.5 per cent. The standard Kahn was not stronger than the Wassermann in any of the untreated cases. In the treated cases the Wassermann exceeded the strength of the standard Kahn in 70 cases or 77.7 per cent, while the Kahn was stronger than the Wassermann in one case or 1.1 per cent.

Comparing the Wassermann and the modified Kahn reactions, we find that the modified Kahn had one false positive reaction in the nonparetics so that 2.08 per cent of those cases had stronger precipitation reactions than complement fixation. In the untreated cases there was a perfect agreement between the two tests, while in the treated cases the Wassermann was the stronger in 28 cases or 31.1 per cent. The modified Kahn was stronger than the Wassermann in only 6 cases or 6.6 per cent of the same group.

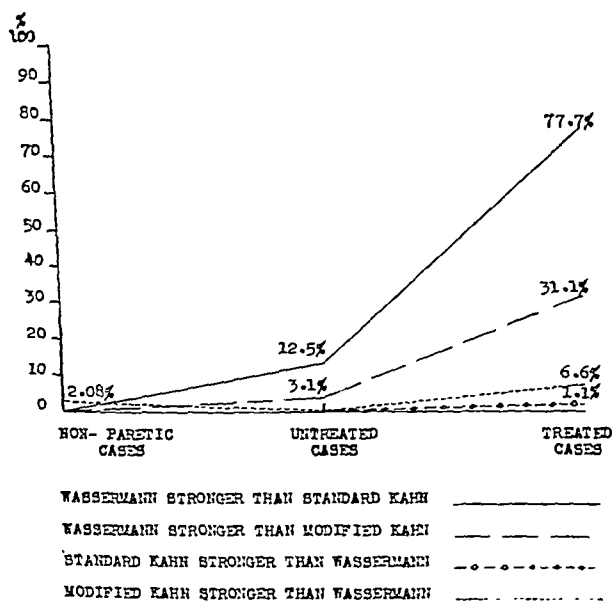


Chart 4.—Comparative strength of the spinal fluid Wassermann and Kahn reactions.

DISCUSSION

It will be noticed from the preceding data that our findings are in great disagreement with other authors. We feel this is due to the great number of treated cases included in our survey, because if only the nonparetic and untreated parietic cases are considered, there would be a relatively high percentage of agreement between the reactions. (Perfect agreement between Wassermann and standard Kahn, 89.2 per cent; Wassermann and modified Kahn, 95.5 per cent.)

Unfortunately the amount of fluid submitted for examinations was not great enough for us to follow completely the suggestion of Kurtz and Larkum and concentrate the globulin to a further extent than that of the standard Kahn technic. Undoubtedly, we would have found as they did, that more positive and stronger reactions could be obtained. However, in view of the fact that

we found one false positive among the 48 cases of nonparetics with the modified Kahn procedure, it would suggest that upon further concentration of the globulin, these false reactions would be found in even greater numbers. We feel that this method would be a dangerous procedure unless carefully controlled by a recognized Wassermann test and clinical evidence.

It is worthy of note that our findings indicate a false impression is prevalent if the Kahn reaction alone is used in determining the success of the treatment of neural syphilis: so many of these fluids had negative Kahns, although the Wassermann reactions remained positive. We definitely recommend the latter as the more delicate and reliable test upon spinal fluids.

CONCLUSIONS

1. The Wassermann reaction (Craig) upon spinal fluid gives a higher percentage of positive reactions in treated and untreated cases of paresis than the standard Kahn or the modified Kahn employing the serum antigen titer.

2. A new method is given to remove the excess ammonium sulphate from the precipitated globulin, which we feel is as efficient and more simple than the method of Kurtz and Larkum.

3. The agreement between the three reactions is relatively high in nonparetics and untreated paretics, but falls off rather sharply in treated cases.

4. The modified Kahn reaction employing the serum antigen titer gave a higher percentage of positive reactions in treated and untreated cases of paresis than the standard Kahn test.

5. One false positive reaction was observed among 48 nonparetics with the modified Kahn reaction, indicating a possibility of error if this test is used exclusively.

We wish to take this opportunity to thank Dr. Sidney D. Wilgus, Director of the State Psychopathic Institute, for his interest, helpful guidance and suggestions in the preparation of this study.

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SPECIAL RACK FOR SMITH FERMENTATION TUBES AND "U" TUBES*

PAULINE EPSTEIN, NEW YORK, N. Y.

ONE of the means of identification and differentiation of various strains of bacteria is the sugar reaction test, for which a Smith fermentation tube is used. The latter is filled with sugar broth into which the organism to be tested is inoculated. The amount of gas produced by the organism as measured by its volume in the arm of the tube, as well as the production of acid, as

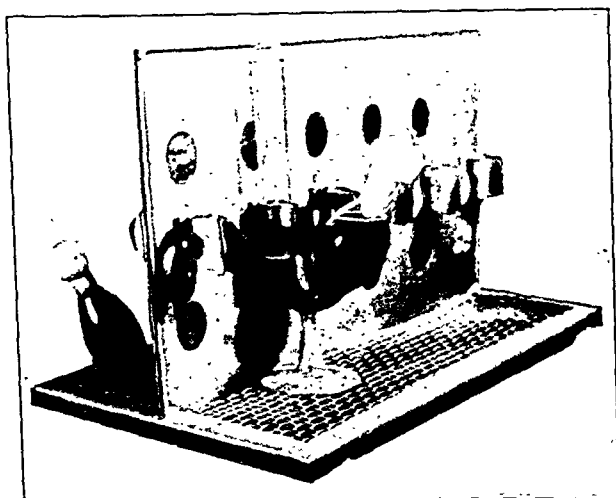


Fig. 1.

evidenced by the change in the color of the sugar broth, frequently facilitates the classification of different microorganisms. However, due to its specific and cumbersome form, the wide use of the Smith tube has been found difficult.

In the process of the fermentation test the tube is subjected to numerous manipulations:

- A. Sterilization of the tube itself in hot air sterilizer.
- B. Filling it with culture media and sterilization of the media in the autoclave or Arnold.
- C. Inoculation with the organism to be tested and incubation for its growth, etc.

In the course of this handling the tube, because of its thin stem, too often breaks. In addition, due to its instability, the tube frequently tilts or falls and displacing part of the gas in the arm by the broth, disturbs the test. To avoid the breakage and to secure the results of the test, I devised a rack which

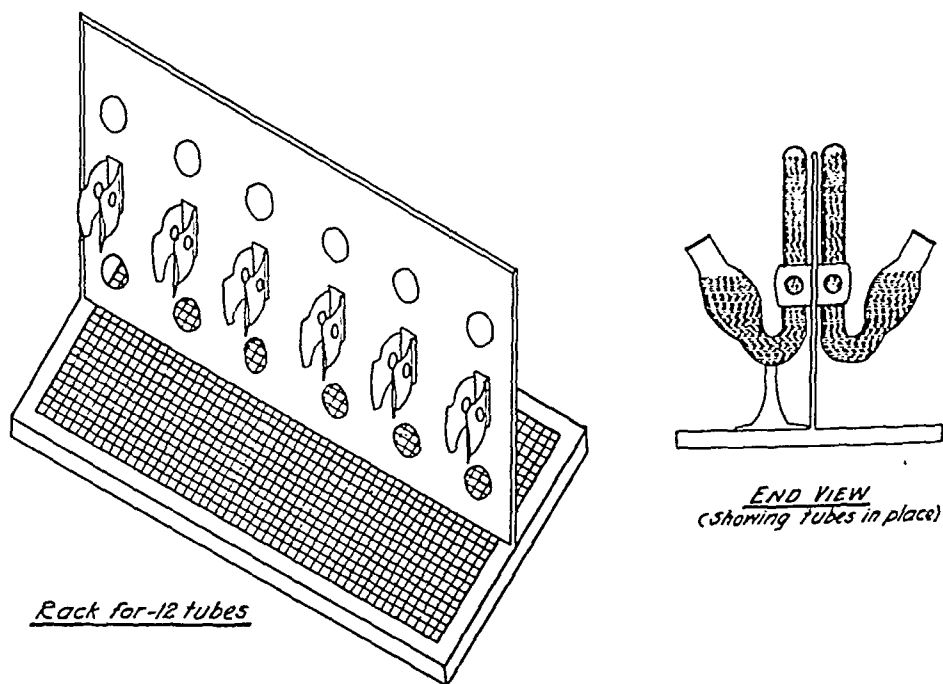
*From the Department of Bacteriology, New York University and Bellevue Hospital Medical College.

Received for publication, April 10, 1933.

has been successfully used for some time in our laboratories. This rack consists of a base and a vertical plate. The base, 13.5 inches by 7.5 inches, is made of a metal frame and a wire mesh, which has 16 openings per square inch, thus allowing free circulation of steam or heated air during sterilization.

The vertical plate, 7.5 inches by 13.5 inches is centered at the base. Attached to the plate, centrally located, are six clasps at $2\frac{1}{4}$ inches spacing on each side.

Directly above and below each clasp there is a $\frac{3}{4}$ inch diameter opening punched through the plate. The sides of the clasps also have $\frac{1}{4}$ inch diameter



openings. The holes in the plate as well as those in the clasps enhance further circulation of air and the maintenance of a uniform temperature through the entire surface of the rack.

The rack is designed to hold "U" tubes as well as Smith fermentation tubes. In either case, the long arm of the tube is placed in the clasp, while in the Smith fermentation tube the stand rests on the base of the rack, thus affording a double support, as shown on the end view of the rack.

Monel metal was used in the construction of the rack because it is rust-proof and preserves its appearance. It is a solid unplated metal, white, very hard and can therefore be subjected to various temperatures.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

DERMATITIS, Contact, Patch Test in the Diagnosis of, Ayres, S. Jr., and Anderson, N. P. *Ann. Med.* 6: 1161, 1933.

The advantages of the patch test in determining the etiology of certain cases of dermatitis are mentioned, together with a description of the method of applying the test.

Twenty-four cases of eczematoid dermatitis are reported, some of them in detail, in which the etiology was established by means of the patch test.

The method advocated by the authors is as follows:

If the substance is a chemical it should be applied in approximately the same dilution to which the patient has been previously exposed. Leaves of plants and shrubs should be as fresh as possible. Dry powders may be moistened slightly with water. The substance to be tested is placed on a small square of cotton about the size of a postage stamp which is then applied to the flexor surface of the forearm, or other suitable area, and covered with a square of some impervious material such as cellophane, which should be about four times as large as the test area. This external covering is then held firmly in place by wide strips of adhesive tape. The space on all sides between the edges of the central cotton square and the edges of the cellophane is sufficient to prevent the adhesive tape from coming too close to the test area. Otherwise the mild temporary irritation which usually results from the adhesive tape might interfere with an accurate interpretation of the reaction. The patch test is allowed to remain undisturbed for twenty-four hours unless discomfort from irritation justifies its removal before this time. A positive reaction is characterized by a sharply defined square of redness corresponding in size and shape to the central test area. Papules and vesicles are also usually present, corresponding to the appearance of the original eruption. Evidences of the reaction can usually be noted for three or four days or longer after the test is applied. In rare instances the reaction may not develop for forty-eight hours after the test is applied.

The test is simple, inexpensive, and closely simulates the natural conditions under which the dermatitis developed. It is usually very accurate. A few cases have been reported in which only certain areas of the skin were hypersensitive so that in the case of a dermatitis of the face, a patch test on the arm was negative while one performed on the face after the eruption had subsided was strongly positive. Such instances are probably uncommon, but the possibility should be borne in mind.

BLOOD PROTEINS, A Micromethod for the Quantitative Estimation of, Medes, G. *Am. J. Clin. Path.* 3: 439, 1933.

One and five-tenths cubic centimeters of blood from the ear of a rabbit is allowed to drip directly into a centrifuge tube containing powdered potassium oxalate, with which it is thoroughly mixed. The tube is centrifuged at 3,500 revolutions a minute for four minutes. The plasma is syphoned off through a fine glass syphon into a small straight-sided vial containing a 2-holed rubber stopper through one hole of which passes the syphon, and through the other, a short glass tube connected by rubber tubing to a glass mouthpiece. By this arrangement the stream of plasma through the syphon can be regulated and the plasma removed from the mass of cells to almost the last drop. The plasma is then distributed to previously prepared 10 c.c. volumetric flasks by means of a Folin tube for micro determination of blood sugar, accurately graduated in 0.1 and 0.2 c.c. graduations.

Preparation of Flasks: The following amounts of anhydrous Na_2SO_4 are weighed on an analytical balance into a series of 10 c.c. flasks: (1) none, (2) 1.07 gm., (3) 1.42 gm., (4) 1.78 gm., (5) 2.204 gm. Into Nos. 2, 3, 4, and 5 are added 0.4 c.c. of a buffer solution to be described below. To all five tubes is added water at 35° to just below the 10 c.c. mark.

The flasks are stored in an incubator at 35° in order to maintain the Na_2SO_4 above its eutectic point. When ready for use, 0.1 c.c. plasma is measured into each of Nos. 1, 2 and 3, and 0.2 c.c., into Nos. 4 and 5, the volumes made up to 10 c.c. with warm water and the flasks returned to the incubator for three hours.

Composition of Buffer (Berglund): 29.6 c.c. of 0.2 M carbonate free NaOH, 50 c.c. 0.2 M of KH_2PO_4 , distilled water (carbonate free) to make 100 c.c. This should give a solution with pH 7.0

At the close of the three-hour period, the contents of Flasks 2, 3, 4, and 5 are filtered. A fine smooth filter paper 6 cm. in diameter is employed. The filtrates are collected in graduated centrifuge tubes; 9 c.c. can usually be procured. At the same time 9 c.c. of the contents of Flask 1 are transferred to centrifuge Tube 1. To each of the five tubes is added 1 c.c. of 50 per cent trichloroacetic acid and the contents thoroughly mixed by means of a fine glass rod which is then washed off with 4 per cent trichloroacetic acid. The centrifuge tubes are allowed to stand for ten minutes at room temperature, and are immersed for ten minutes in a water-bath at 50° C. They are centrifuged at 3,500 revolutions per minute for twenty minutes, after which the supernatant fluid is decanted and the precipitate dissolved in one or two drops of 10 per cent NaOH stirring thoroughly with a fine glass rod and spreading the NaOH over the entire inner surface of the centrifuge tube. The contents are then made up accurately to the original volume (9 c.c.).

The nitrogen of each of these solutions is determined by Folin's micro Kjeldahl method, employing 2 c.c. of each. Occasionally 3 c.c. may be required for some of the lower fractions of globulin.

Computation:

$$S \times \frac{20}{R} \times \frac{10}{X} \times \frac{100}{Y} = N$$

where S = value of standard, R = reading of colorimeter, X = 0.1 or 0.2 c.c., according to the fraction being ashed, Y = No. of c.c. ashed, and N = milligrams protein nitrogen in 100 c.c. of plasma. To obtain the grams of protein per 100 c.c. of plasma, multiply by 6.25 and divide by 1,000. Total protein equals protein in Tube 1; fibrinogen equals Tube 1 minus Tube 2; euglobulin equals Tube 2 minus Tube 3; pseudoglobulin I equals Tube 3 minus Tube 4; pseudoglobulin II equals Tube 4 minus Tube 5; albumin equals Tube 5. In case values of only total protein, fibrinogen, globulin and albumin are desired Tubes 3 and 4 are omitted.

MALARIA, A Method of Making Slide Smears From Female Anopheles, etc., Barraud, P. J. Ind. J. Med. Res. 21: 457, 1933.

The advantages of the technic described may be summarized as follows:

1. The method combines, in the large majority of preparations, the advantages of dissection of the salivary glands, with those of Sergeants' method for detecting the presence of sporozoites in the body-fluid of mosquitoes.

2. The insect host is preserved for identification and for reference in the future.

3. The method can be carried out rapidly in the field where laboratory facilities, and sufficient time, for carrying out dissections in the ordinary way, are not available.

4. The preparations can be made by an assistant not necessarily trained in the detection of sporozoites, or in the identification of mosquitoes.

5. A microscope is not necessary for making the preparations.

The apparatus required is as follows: A supply of test tubes with wool plugs; a supply of microscopical slides; two needles, or pins (preferably mounted in handles); some staged tubes; a supply of small double-pointed entomologic pins and a pair of entomologic forceps. A chloroform tube, or a supply of chloroform, is also useful. The slides may be prepared previously by marking off three spaces by drawing transverse lines with a diamond, about one inch apart, and numbering these consecutively.

The mosquitoes are caught in test tubes. When a preparation is to be made, kill the

insect with chloroform, or by blowing tobacco smoke into the tube. When dead, turn the specimen out on to a slide. Arrange the whole mosquito on a numbered square, on its left side, with the head pointing away from you. Do not add any saline, or other fluid, as this is not required. Do not remove the wings or legs, and endeavor to carry out the following manipulations with as little damage as possible to the scaling, etc. Take a needle in the left hand and place the shaft of this flat across the front of the thorax. Press down with this so as to cause the thin chitin of the front of the thorax below the neck ("prosternum") to bulge out considerably.

The salivary glands lie just within this thin sheet of chitin. With a needle held in the right hand cut into this part. Draw out, and cut off, the tissues which will protrude, and by continued pressure with the left hand needle at the same time, squeeze out some of the body fluid on to the slide. Endeavor to avoid severing the neck, or detaching the front legs. Tip the mosquito on to a piece of paper, and with a needle rapidly mash up and make a small circular smear, on the numbered square, of the tissues and fluid left on the slide. Dry this by waving the slide in the air. It should be noted that the dissection and smear are made without any admixture of saline. When opportunity occurs the smear should be fixed with methyl alcohol or absolute alcohol, and the slides stored for staining and examination, which may be done at any convenient time later.

Pin the mosquito and mount it in a staged tube, marking on the stage the number corresponding to the smear on the slide. Observations on the locality, the nature of the place where the specimen was collected, the date, and any other details considered worth recording, should be entered in a notebook at the time.

Before making another preparation clean the needles thoroughly.

GNOCOCCI, Cultivation of, Philadelphia, A. *Wien. Klin. Wchnschr.* 46: 1052, 1933.

Two parts sterile human or horse blood are mixed with one part of distilled water in a flask. The flask is closed with a sterile glass stopper and shaken vigorously for three minutes to prevent coagulation. It is then kept in the incubator at 60° for half an hour. After this time a foamy mass of fibrin collects at the top, while the dark red blood serum is fluid. This is mixed in a proportion of 1 to 2 with nutritive agar at 60° with a 2 per cent agar content and a P_H of 7.3 to 7.5. The nutritive medium is ruby red and transparent.

The author made 415 cultures from 108 patients suspected of gonorrhea. In 354 instances the cultures agreed with the microscopic findings. In 29 instances the cultures showed growth of gonococci though no gram negative gonococci were found microscopically. In four cases that were doubtful microscopically cultures gave positive results. In three cases that were positive microscopically there was no growth of the cultures. The results of cultivation on blood serum agar were at least as good as those on ascites agar.

IODINE, An Improved Micromethod for Estimating, Patnaik, M. *Ind. J. Med. Res.* 21: 237, 1933.

The sample for analysis is rendered alkaline. Fats and oils should first be saponified with 10 per cent alcoholic potash, then evaporated to dryness in a porcelain basin and dissolved in hot water. Cereals and vegetables should be heated with alkali till a complete paste is obtained. Whatever the sample may be it should be converted into an easily workable form. To such an alkaline solution 7 per cent solution of $KMnO_4$ is gradually added and while stirring with a glass rod the porcelain basin is heated over an asbestos protected flame. When the oxidation is complete and there is a slight excess of $KMnO_4$, absolute alcohol is added slowly until the excess of $KMnO_4$ is reduced. It is then cooled and filtered quantitatively and the hydrated manganese dioxide removed. The filtrate is then evaporated to dryness with a small amount of K_2CO_3 . The residue which always contains some nonoxidizable disintegration product, besides potassium iodate, is heated over a protected flame till charring takes place without the evolution of smoke (some amount of smoke is evolved from fats and oils at this stage—the amount of smoke being very much less than that in the usual incineration method). It is then heated slowly over a mild flame till the organic matter is

disorganized. During this process of heating, all the iodate coming in contact with the finely divided carbon is reduced to iodide. It is then made into a paste and extracted and estimated as usual.

If during incineration the charred mass remains in a molten state due to an excess of potash, a requisite amount of K_2CO_3 should be added to render the final product suitable for alcoholic extraction.

For the determination of iodine from pure $KMnO_4$, 50 gm. of $KMnO_4$ is taken, and after reduction with alcohol it is filtered quantitatively. The filtrate is evaporated in five nickel dishes after addition of 3 gm. of K_2CO_3 to each dish. Then before the evaporation is complete one gram of sugar (there being no iodine in sugar) is distributed among the five dishes. The rest of the process remains the same. In every determination the amount of iodine present in the $KMnO_4$ used up is subtracted in order to get the actual value.

SYPHILIS, Flocculation Tests For, Mudge, E. Am. J. Clin. Path. 3: 421, 1933.

The following tests were compared in 1,000 blood specimens: Flocculation tests: Eagle, Hinton, Kahn, Kline, Meinicke (M.K.R.), and Miller (M.B.R.). Complement fixation tests: Kolmer quantitative, and a double antigen test.

This series has shown the advantage of performing a complement fixation test and a flocculation test on every blood specimen as supplemental to each other.

The flocculation tests have a definitely higher degree of sensitivity than the complement fixation tests in treated and latent cases of syphilis.

The majority of the tests concerned have a high degree of specificity.

All eight tests require a high degree of technical skill, and increasing experience with any test enhances its value.

SERUM PROTEINS, Diagnostic Value of Changes in Kala Azar, Mudaliyar, M. R. G., Sundarum, S. K., and Ramachandran, A. S. Ind. J. Med. Res. 21: 361, 1933.

The serum protein changes in kala-azar while not absolutely diagnostic of the disease offer distinct help in diagnosis of kala-azar in doubtful conditions.

The essential changes in serum proteins in kala-azar are a fall in albumin with considerable rise in total globulin, associated with increase in the relative proportion of euglobulin. A similar, but less striking change, has previously been recorded by Lloyd in secondary syphilis.

Serum protein changes in a number of miscellaneous conditions have been recorded.

B. PERTUSSIS, Rapid Agglutination Technic Applied to, Kendrick, P. L. Am. J. Pub. Health 23: 1310, 1933.

Antigen.—The forty-eight-hour growth of *B. pertussis* on a Bordet-Gengou slant is transplanted to a half Petri plate of Bordet-Gengou medium. After forty-eight hours' incubation, the growth is removed with a stiff, bent needle and emulsified in 1.5 c.c. of physiologic salt solution. The suspension is filtered and adjusted, if necessary, to a turbidity of approximately 10 billion organisms per c.c. by comparison with a standard.

Filtration is an important step in obtaining satisfactory smooth suspensions. Test tube filters are prepared for this purpose. A thin layer of moistened cotton is shaped around the finger, placed on a simple copper wire netting support, and inserted in a $5 \times \frac{5}{8}$ tube, the free end of the wire strip being bent to hook over the lip of the tube. The cotton is molded against the walls of the tube by means of a wooden applicator. These filter tubes are plugged with cotton and sterilized. The filter is easily removed after use and the plug reinserted.

For a larger filter, a layer of gauze and cotton is wrapped around the outside of a square of wire netting cut from a bias strip and shaped to fit inside the neck of the bottle or flask of the desired size. The filter is suspended by two wires which cross under it, pass through the netting, and are hooked over the lip of the flask.

Antiscrum.—For preparation of a diagnostic serum, a rabbit is injected intravenously with a 10 billion per c.c. suspension prepared from a forty-eight-hour growth of a recently isolated *B. pertussis* culture to which merthiolate 1:10,000 has been added at least twenty-four hours previously. The injection doses are 0.4 c.c., 0.8 c.c., and 1.0 c.c., respectively, at three- to four-day intervals. About 1 week after the last injection, the agglutination titer (equivalent, as explained below) has been around 1:20,000 in the six different rabbits treated by this method. The serum dilutions for agglutination tests are chosen according to the particular conditions. For testing cultures isolated by the cough plate method, the dilutions usually employed are 1:10, 1:100, 1:500, 1:750, 1:1,000, 1:1,500, and 1:2,000. For uniformity, the same dilution scheme is always employed and separate pipettes are used for the different dilutions.

The agglutination test—one-tenth c.c. of each serum dilution is mixed with 0.1 c.c. of antigen, the measurements being made with 1 c.c. pipettes graduated in tenths. For an antigen control, 0.1 c.c. of saline is mixed with 0.1 c.c. of antigen. The mixtures are shaken by hand for three minutes. For the shaking process, the racks are rocked at the rate of approximately 60 back-and-forth motions per minute and in such a way that the contents flow up the walls of the tubes for about three-quarters of their length. After the shaking period, physiologic salt solution is added for greater ease in reading. Because of its convenience, the Hipple pipetting apparatus, used in the Kahn test and set to deliver 0.5 c.c. of saline, is ordinarily employed.

Reading the tests—the tests are read immediately after the addition of saline and each tube recorded as —, ±, +, ++, +++, or +++++, according to the degree of agglutination. In the interpretation of the results in comparison with those given by other workers, a question arises because the final dilutions in the rapid test are not strictly comparable with those ordinarily given. This is of small consequence, however, provided the method of expressing the results is clearly stated. In the 0.2 c.c. mixtures of the rapid test, the series of serum dilutions 1:10 to 1:2,000 would give a series of final dilutions from 1:20 to 1:4,000. Any particular final dilution in the usual 1 c.c. test would contain five times the actual quantity of serum and antigen contained in the same dilution of the 0.2 c.c. rapid test. Therefore, if based on the actual quantity of serum present in the mixture, the series of final dilutions 1:20 to 1:4,000 of the rapid test would be equivalent to a series 1:100 to 1:20,000 in the usual 1 c.c. test.

TYPHOID BACILLI, Isolation of From Water and Sewage, Wilson, W. J. Brit. M. J. 3794 (Sept. 23), 560, 1933.

The method following is described as very efficacious:

To 100 c.c. of 3 per cent nutrient agar were added 10 c.c. of a stock glucose-phosphate-sulphite-bismuth mixture, and 1 c.c. of a 1 per cent solution of brilliant green. Equal volumes (25 c.c.) of the medium, cooled to 55° C., and water were mixed and poured into Petri dishes. After the medium had set and been incubated, black colonies consisting of typhoid bacilli developed in its substance. These were subcultured on to MacConkey plates, and cultural and serologic tests applied.

The bismuth media are very easily prepared when a stock solution is available, and such stock solutions keep for months. Dissolve 6 grams bismuth-ammonio-citrate scales in 50 c.c. of boiling distilled water, and neutralize by the addition of about 2 c.c. of 10 per cent caustic soda. Mix with a solution obtained by boiling 20 gm. sodium sulphite anhydrous in 100 c.c. of water, and then, while the mixture is boiling, add 10 gm. of sodium phosphate anhydrous. To the sulphite-bismuth-phosphate mixture, when cool, add a solution of glucose obtained by dissolving 10 gm. of commercial glucose in 50 c.c. of boiling distilled water. For preparation of old standard medium, add to 100 c.c. of a hot melted 3 per cent nutrient agar 20 c.c. of stock mixture, then 1 c.c. of an 8 per cent solution of ferrous sulphate crystals in water, and 0.5 c.c. of a 1 per cent solution of brilliant green in distilled water; pour into Petri dishes, and when the medium has set inoculate the surface. Isolated colonies of *B. typhosus* and *B. para-typhosus* are black, the typhoid colonies usually appearing within twenty-four hours, and the paratyphoid within forty-eight hours.

UNCINARIASIS, Acute From Massive Infestation and Its Implications, Ashford, B. K., Payne, G. C., and Payne, F. K. J. A. M. A. 101: 843, 1933.

In the discussion of an outbreak following prolonged bathing in polluted water the authors comment that the secret of what happened to these patients is found in a study of the leucocytes.

First, infestation, when sufficiently massive, produces leucocytosis, and this leucocytosis is eosinophilic. In every one of these cases this occurred and on these abnormalities, with a history of ground-itch, sudden loss of strength and color, and, generally, diarrhea, the correct diagnosis should be strongly suspected, if not actually made clinically, as in this instance it was made. In these cases, in from one to two months after infestation, a high leucocytosis was found already developed. It oscillated for two or three months and then, throughout nine months, gradually sank to near or quite normal.

But if the leucocyte count is striking, the eosinophile percentage is still more so. When the patients were first examined, this percentage was found to lie all the way between 14 and 69. When most of the worms in the intestine had been expelled, the percentage arose; but this was not on account of the expulsion of the worms. There is, we believe, a more reasonable explanation: There is a growing feeling that eosinophilia is a phenomenon related to the disintegration and absorption of foreign protein. The old idea that eosinophilia in uncinariasis is due to a toxin produced by the worm can no longer be accepted in its literal sense. The eosinophilia rises when the larval mortality in the tissues rises.

BIOPSY, Present Status of, McGraw, A. B., and Hartman, F. W. J. A. M. A. 101: 1205, 1933.

The worth of the biopsy in diagnosis, prognosis and as an index for treatment is at present thoroughly and soundly established.

Properly conducted, its lack of danger is likewise established. Its few contraindications are well known and recorded.

Its status as a technical procedure, though clearly allowing of future improvement, has attained a satisfactory level of combined simplicity in procedure and materials and of reliability in preparation.

Its opportunities for future development and practice under optimum conditions lie largely in stimulation of interest and furnishing of facilities for more pathologists and surgeons to devote the time and hard work necessary to perfect themselves in the difficult field of tumor pathology.

POLYCYTHEMIA, Benign Familial, Spodaro, A., and Forkner, C. E. Arch Int. Med. 52: 593, 1933.

Observations on the blood, together with other clinical studies, of ten members (two generations) of a family in which polycythemia and splenomegaly were present are reported. The existence of polycythemia in an earlier generation of the family is suggested by the history.

This family differed from those previously described in that characteristics of polycythemia vera, other than the increased number of red blood corpuscles and enlargement of the spleen, were not present.

The term "benign familial polycythemia" is suggested to distinguish this condition from polycythemia vera and from polycythemia due to recognized causes.

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EDITORIAL

Some Pitfalls in the Study of Sex Hormones

ONE of the most diligently cultivated fields of scientific investigation at the present time is that of the interrelations between the gonads and the anterior hypophysis, the fruits of which have already amassed a large volume. As is always the case, much of this literature is confusing in its conclusions, with, oftentimes, a blind following of methods already outmoded. In the following paragraphs an attempt will be made to evaluate some of this literature in terms of actual accomplishments, with a view of aiding further work along this line. The whole subject has been amply reviewed by many able investigators and a duplication of this will not be attempted, the aim being rather to correlate certain phases of the work.

As one examines the literature it is evident that the same criteria of the efficacy of administration of the sex hormones is fairly general, the weights of the gonads of immature animals which have received injections for a short period, usually two or three days, with the opening or closure of the vagina. In very

many cases, particularly with routine assaying of extracts, the latter test only is used, many investigators using the same animals repeatedly, with short intervals between each test.

It has been shown (Lipschütz, 1932; Swezy, 1933a; Evans et al., 1933; Fluhmann, 1933) that the ovary has no necessary relation to the vagina when hormones are administered experimentally, cornified cells occurring with the ovary in all stages of change between normal and excessive luteinization, and with or without large follicles, hence this test gives evidences only of the presence of the single factor responsible for vaginal changes. Lipschütz further showed that these vaginal changes may take place with no corresponding changes in either uterus or ovary. Much evidence is also accumulating to show that this factor is not identical with folliculin, progesterin, the growth, follicular growth, or luteinizing hormones, and is widely distributed, occurring even in plants. As was first demonstrated by Stockard and Papanicolaou (1917), the different stages of the normal estrous cycle of many of the lower mammals are characterized by clear-cut changes in the cellular contents of the vagina. Much experimental work has also shown that these changes are directly due to the action of hormones. The work shows equally well, however, that decreasing or increasing these hormones completely upsets the normal relations between them, resulting in conditions which diverge widely from the normal, not only in the ovary, but in the uterus, vagina, and even in the hypophysis itself. Under such conditions it is too much to expect that the vagina will still reflect the changes in the ovary. That this is true is abundantly proved by the results of much published work, in which all conditions of the ovary are associated with estrous smears as well as with diestrous ones. This fact that it does not give reliable information concerning the state of the ovary and uterus makes the vaginal smear method an unsuitable one for sole reliance as an assay test for sex hormones. In his recent review of the biologic assay of the sex hormone of the ovary, Marrian (1933) omits all mention of any other test for this hormone. That it is the sole test in so much of the work with hormones may well account for much of the variable results reported.

The practice of using the same animals repeatedly after a few days' interval has an even greater fault. The recent work of Emery (1933) shows that the ovaries of rats receiving injections of two castrated rat hypophyses on two successive days, reach a maximum size at twelve days and remain larger than the controls for about two months, when rats twenty-five days old are used. Since this is the usual age and length of treatment for experimental rats, it is evident that they will not be in optimum condition for some time after the initial treatment, as it is doubtful if results exactly comparable to those obtained in animals with ovaries in the normal immature state will be obtained in animals with ovaries already extensively luteinized. Even when no vaginal changes are found in these animals, it has been shown that changes in both ovary and uterus may take place (Evans et al., 1933), which might render their use a few days later of questionable value. The results reported by Selye and Collip (1933) show that early luteinization (day sixteen) will prevent the usual reaction of the ovary at a later date (day twenty-six) with continued injections. The work of Evans indicates the same possibility. The use of such animals may account for

much of the variable results obtained from different samples of these hormones put out by commercial laboratories, but the practice is by no means confined to such laboratories, as it is usually recommended for all routine work. Coward and Burn (1927) used animals not more frequently than once a fortnight, while Allen et al. (1930) used rats once a week. Coward and Burn found that out of 50 rats used three times, only 19 gave the same result each time. These were ovariectomized. Since these hormones affect the ovary mainly, it is probable that the differences would have been greater in normal animals. It is doubtful whether the disadvantages of using animals repeatedly, without a sufficient time elapsing for full recovery, can be compensated for by using large numbers of animals. This might be of little importance with many experiments, but for careful analytical work it is the attention to such small details which assures success.

The use of the weight of the ovary as an assay test is also open to criticism, since this is no infallible guide to the kind of change induced by hormone treatment, though excessive weights are nearly always associated with extensive luteinization. In a recent article Van Dyke and Wallen-Lawrence (1933) attempt to determine the differentiation of the follicular growth and luteinizing hormones by the weights of the ovaries induced in immature rats, without reference to the possible presence or absence of lutein cells in them. It is necessary that some follicular growth take place before a noticeable change in the weight of the ovary is obtained. But, since the luteinizing hormone may induce the formation of lutein cells from the germinal epithelium and tunica albuginea (Swezy, 1933a), it seems certain that evidences of the presence of this hormone may be found with but little change in either size or weight of the ovary. Selye et al. (1933) induced luteinization in the thecal cells of the small follicles of the ovary of the rat, beginning about day thirteen, with no macroscopic change in the ovary, with pregnancy extracts, and in the guinea pig without accompanying follicular growth. Any attempt therefore to determine the presence or absence of the luteinizing hormone in any preparation would involve a careful examination of stained sections of the ovary, even though no response had been elicited by the treatments in the vagina or in the weight of the ovary. In fact this seems more necessary in the case of the latter animals, as a pure luteinizing hormone would thus be demonstrated.

A considerable amount of follicular growth may also be found without a significant increase in the weight of the ovary. The presence of follicular growth hormone in small amounts could be detected only by the same means as has been suggested for the luteinizing hormone. Between this stage and that of extensive luteinization or the production of large follicles in great numbers, however, it is not always possible to determine from macroscopic examination what structures are predominant.

The relation of estrin, or factor inducing cornification in the vagina of rodents, to folliculin, or factor inducing growth in the endometrium, is one fraught with much confusion. In the normal animal these two factors seem to occur at approximately the same time in most cases, though this does not always hold true in the cat (Evans and Swezy, 1931). Under experimental conditions the preponderance of evidence indicates that the factor inducing cornifi-

cation is not identical with that inducing growth in the uterus, though the idea that they are one and the same seems to be prevalent. This acceptance of their identity has resulted in the widespread use of the vaginal smear test as the important one in the assay of these hormones.

Many investigators believe that a single hormone may be responsible for the changes in uterus and vagina, the relation being a quantitative one, yet, *in the normal animal, maximal growth of the endometrium is associated with cornification, this never occurring during the initial stages of growth when a smaller amount of hormone is presumably present.* Further evidence against this assumption is found in the work of Evans et al. (1933), in which all stages of growth of the uterus from medium to an estrous type and ovaries with medium and large follicles are present in rats treated with hypophyseal hormones and the vagina still closed. On the other hand, Lipschütz obtained vaginal changes with the administration of hypophyseal extracts but no changes in either ovary or uterus. The presence of cornification with no medium or large follicles, has been noted by a number of investigators, as well as ovaries filled with large follicles and a diestrous smear in the vagina. With these results in both normal and experimental animals, there seems to be no grounds for assuming that a single factor is involved in the changes in the vagina and uterus. Two separate factors are indicated.

Allen et al. (1930) found that the amount of estrin present in human corpora was highest after recent ovulations, days thirteen to sixteen, and lowest in ovaries with older corpora, in two cases at days twenty and twenty-two, and that there was an abrupt drop with menstruation. It is well known that in the first half of the ovarian cycle following ovulation, there are no large follicles in the ovary, these reaching a large size only toward the end of the cycle. Since ovulation seems to occur in the middle third of the cycle in the majority of cases, these relations would mean that the eight to ten days preceding menstruation would find only small to medium-sized follicles in the ovary. Frank and Goldberger (1926) find, however, that estrin reaches its greatest concentration near the first day of menstruation, the early part of the cycle showing only a small amount, with an increase from day ten to fifteen. If these were not cases in which ovulation occurred near menstruation, these results would suggest that the greatest concentration of follicular secretions came at a time when there were apt to be only small to medium-sized follicles in the ovary. This assumption is contradictory of much work on the lower mammals, since here the greatest concentration, as shown by the only critical test, the growth of the uterus, comes immediately preceding or coincident with ovulation. At first sight this would seem to mean that these physiologic processes and hormones differ radically in the human from those of the lower mammals, yet a considerable body of work indicates that they are very similar. Frank and his coworkers used only the presence of cornification in the vagina as the test of these hormones.

Cornification has no recognized place in the human cycle, hence the factor for it, found in a wide range of preparations, including plants, would seem to have no invariable relation to the ovarian cycle, while the factor causing growth in the endometrium does have a constant and invariable relation. The regularity of the uterine cycle in the human being, with its dependence on folliculin, is as

constant as may be found in the rat or guinea pig. There are no evidences yet brought out indicating a different hormonal relation. A clarification of these relations and the hormones associated with them can be obtained only by more critical analyses of the experiments conducted with them. In the light of present data it would seem expedient to restrict the use of the term estrin to the factor related directly to the vaginal changes, and, what is more important, to restrict the vaginal smear test to the assay of estrin, and not use it as a valid test for other hormones.

It has been pointed out elsewhere (Swezy, 1933b) that in all experimental work on normal animals, the hypophysis of the test animal itself largely decides the results when sex hormones are being studied. Whether this is true of other hormones also must be determined by further work. This result seems to be due to the presence of an activator in some preparations, including human pregnancy extracts and the hypophysis of the infantile rat, which is able to stimulate the hypophysis of the host to increased hormone production. This conclusion is supported by a number of experiments from different laboratories. The difficulties of determining the hormone content of any preparation are thus increased, since this can be done accurately only when the animal's own hypophysis is eliminated through hypophysectomy. This does not disparage the use of normal animals for such experiments, however, as these are a vital necessity in gaging the therapeutic value of such preparations, but it does suggest the necessity of basic work under carefully controlled conditions, and such conditions are not obtainable in the normal animal, its own hypophysis being the disturbing factor.

In judging the value of these preparations the "gonad-stimulating potency" has been one of the main tests. In the rat this potency is measured by the weight of the ovaries produced, great potency producing heavy ovaries, filled mainly with luteal tissue. The luteinizing hormone, which brings about this result, checks ovogenesis and the growth of follicles (Swezy, 1933a) and produces ovaries having a tumor-like metabolism (Szarka et al., 1933). In the normal animal the corpora lutea, so far as present data indicate, have little or no function beyond inducing the secretion phase in the uterus and the maintenance of pregnancy. It is therefore a serious question of what value the administration of the luteinizing hormone, which has, as its chief function, the induction of the formation of this luteal tissue, may be in the nonpregnant individual. Its disadvantages would seem to outweigh any possible benefits. On the other hand, the hormone which stimulates the growth of the follicles, resulting in the secretion of folliculin with its reaction on the hypophysis itself, would seem to have a greater value as a therapeutic agent. This aspect of the effects of the "gonad-stimulating hormones" needs further study before the clinical importance of these preparations can be adequately tested, and some other criterion must be found for evaluating the extracts which will not stress the luteinizing hormone as the important one, a position which it holds in present methods of testing them for potency. In this connection the suggestion of Leonard (1933), that pregnancy extracts should be combined with the hormones of the hypophysis when "great ovarian stimulation" is desired, further emphasizes the need of caution on this point, as the desirability of ever producing such great ovarian

stimulation is open to very grave doubt. These heavy ovaries contain abnormal masses of luteal tissue, derived mainly from atretic follicles, and thus represent a pathologic condition which would seem to have little therapeutic value.

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—Olive Swezy.

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CLINICAL AND EXPERIMENTAL

THE COMBINED EFFECTS OF BILE SALTS AND OLEIC ACID ON CHOLERESIS*

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THE word "choleresis" is here used in the sense advocated by Brugster and Horster.¹ It stands in the same relation to the secretion of bile that the word "diuresis" does to the secretion of urine. A substance that increases bile production is, according to this terminology, called a diuretic as contradistinguished from a cholagogue, a substance that promotes the expulsion of bile.

That bile salts, when absorbed, stimulate the further production of bile, has been established beyond reasonable doubt by Schiff,² Rosenberg,³ Okada,⁴ Foster, Hooper and Whipple,⁵ Specht,⁶ Mellanby,⁷ and numerous others after them, prominent among the recent workers being Chabrol⁸ and his collaborators.

The work of Doyen and Dufourt,⁹ showing that soap causes a slight diminution in the bile secretion during the first twenty-four hours, prompted Okada⁴ to investigate the effect of sodium oleate as a cholagogue. He found that sodium oleate, after ingestion, exerts quite a powerful stimulating effect on bile production. Chabrol and Charonnat⁸ found that oleic acid as well as sodium oleate administered both intravenously and into the duodenum caused a marked choleresis.

As far as could be determined from a review of the literature, little work of any consequence has been done on the potentiation of one cholagogue by another on experimental animals. Meissner¹⁰ investigated the effects on bile

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secretion of salicylic acid and the sodium salt of cinnamic acid and of benzoic acid and theobromine salicylate. Finkelstein and Lipschutz¹¹ showed on human subjects with the aid of the duodenal tube, that when bile salts were injected into the duodenum, the biliary output as compared with control periods averaged 91 per cent; but that when oleic acid was administered with the same dose of bile salts the increase was over 143 per cent. Under their experimental conditions, however, it was impossible to eliminate the cholagogue factor of both the substances used.

The purpose of the work to be reported below is to determine whether relatively small doses of bile salts combined with small doses of oleic acid, cause a larger flow of bile than when these two cholagogues are given separately.

EXPERIMENTAL PROCEDURE

Twenty-four picked animals were used in this investigation. They were all healthy, young adults, all weighing 15 kilograms at the time of the experiment. The food was the stock feed of scraps, used in the laboratory kennels, and given twenty-four hours before the experiment. All the animals were anesthetized with nembutal* (pento-barbital) 750 mg. per dog, given intravenously ten minutes before the operative preparation. In order to insure a uniform anesthesia, each animal was given 100 mg. more of nembutal each hour after the initial dose, until the termination of the experiment.

The operative procedure was similar to that used by Chabrol.⁸ The abdominal cavity was opened through a midline incision. The common duct was cannulated, and the cannula connected with a rubber tubing through a U-shaped glass tube after the method of Rous and McMaster.¹² The rubber tube was then made to project out of the abdominal cavity into the collecting vessel. The gallbladder was segregated by a ligature around the common duct. For the instillation of the cholagogues into the duodenum, a moderately rigid rubber tube was introduced through a small opening on the anterior wall of the pylorus into the duodenum. This tube was anchored to the pyloric wall by a purse-string suture and was just long enough to project out of the abdominal cavity. When not in use, it was kept closed with a screw clamp. The abdomen was closed around the two outjutting tubes at the completion of the operation.

Specimens of bile were collected every half hour until a level or a downward trend in the amounts for three successive periods was obtained before the cholagogue substances were instilled through the tube leading into the duodenum. In some experiments this level or downward trend was attained in four collections, in others, in five, or six collections. Inasmuch as this time variation was evenly distributed in the three series, it was not taken into consideration as a possible source of error.

The collection after the instillation of the cholagogues was continued for three more periods. This was done because in preliminary experiments it was found that under the above experimental conditions and in the doses of cholagogues used, the increased bile flow was evident in the first three half hour

*Grateful acknowledgment is made of the nembutal kindly furnished us by the Abbott Laboratories, New York, New York.

collections after administration, and that in the fourth period the yield was back to nearly that of the period immediately before the administration. The total collection of the three postinstillation periods, was, therefore, deemed to be a faithful representation of the increased choleretic induced by the choleretics. The amounts collected during the three half hour collections prior to the instillation of the choleretics were used as the controls.

The twenty-four experiments were divided into three series of eight experiments each. The first eight dogs were given bile salts in the dosage of 150 mg. each of sodium taurocholate and sodium glycocholate, in 10 c.c. of water. The second series were given 3 c.c. of oleic acid with 7 c.c. of water, and the third series 150 mg. each of sodium taurocholate and sodium glycocholate, plus 3 c.c. of oleic acid and enough water to make up to 10 c.c.

The protocols are reproduced in table form (Tables I, II, and III).

DISCUSSION

It will be seen from Table I that in this series five showed a rise after the administration of bile salts, while three showed a fall. Thus bile salts failed

TABLE I
SERIES I—BILE SALTS*

EXPER.	BILE COLLECTED IN ½ HOUR PERIODS BEFORE ADMINISTRATION			BILE COLLECTED IN ½ HOUR PERIODS AFTER ADMINISTRATION			TOTAL AMOUNT OF BILE COLLECTED IN THREE HALF HOUR PERIODS		PER CENT INCREASE
	3RD PER.	2ND PER.	1ST PER.	1ST PER.	2ND PER.	3RD PER.	BEFORE	AFTER	
	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	
1	2.1	1.5	1.1	1.1	1.4	0.8	4.7	3.3	-29.8
2	1.6	1.5	1.1	1.0	1.7	1.3	4.2	4.0	-4.8
3	1.3	1.1	1.1	2.5	2.4	1.6	3.5	6.5	85.7
4	0.9	0.8	0.8	1.1	1.2	0.8	2.5	3.1	24.0
5	2.3	2.3	2.3	2.6	2.8	2.5	6.9	7.9	14.5
6	1.5	1.4	1.4	2.1	2.7	2.6	4.3	7.4	72.1
7	0.6	0.6	0.6	0.6	1.2	1.4	1.8	3.2	77.8
8	2.2	1.8	1.4	1.4	2.0	0.5	5.4	3.9	-27.8
Average							4.16	4.91	18.0

*150 mg. each of sodium taurocholate and sodium glycocholate administered into the duodenum after the first three collections.

as a choleretic in 37.5 per cent of the experiments. In the former group the highest yield was 85.7 per cent above the control period. The average of the total of the three collections after administration in the eight experiments was 18 per cent above that preceding administration.

Of the eight experiments in Table II, again five showed a rise after oleic acid and three showed a fall. The percentage failure is here the same as in Series I. The highest percentage rise in this group was 100 per cent, and the average of the total of the three half hour collections after the administration was higher by 18.3 per cent than that of the same periods before. It is apparent that the dose of bile salts and of oleic acid used here had about the same choleretic power.

TABLE II
SERIES II—OLEIC ACID*

EXPER.	BILE COLLECTED IN ½ HOUR PERIODS BEFORE ADMINISTRATION			BILE COLLECTED IN ½ HOUR PERIODS AFTER ADMINISTRATION			TOTAL AMOUNT OF BILE COLLECTED IN THREE HALF HOUR PERIODS		PER CENT INCREASE
	3RD PER.	2ND PER.	1ST PER.	1ST PER.	2ND PER.	3RD PER.	BEFORE	AFTER	
	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	
1	0.8	0.6	0.4	0.3	0.3	0.7	1.8	1.3	-27.8
2	0.5	0.5	0.4	0.5	1.0	0.9	1.4	2.4	71.4
3	0.9	0.6	0.6	0.8	0.5	0.4	2.1	1.7	-19.0
4	0.7	0.7	0.6	0.6	0.8	1.0	2.0	2.4	20.0
5	0.6	0.6	0.6	0.6	1.2	1.4	1.8	3.2	77.8
6	2.6	2.0	1.6	2.4	1.4	1.1	6.2	4.9	-21.0
7	1.3	0.7	0.7	1.2	2.1	1.1	2.7	4.4	63.0
8	0.4	0.4	0.4	0.8	1.0	0.6	1.2	2.4	100.0
Average							2.4	2.84	18.3

*3 c.c. of oleic acid in 7 c.c. water.

Table III, which represents the series of eight experiments in which the bile salts and oleic acid were combined, shows some striking figures. All the

TABLE III
SERIES III—OLEIC ACID PLUS BILE SALTS*

EXPER.	BILE COLLECTED IN ½ HOUR PERIODS BEFORE ADMINISTRATION			BILE COLLECTED IN ½ HOUR PERIODS AFTER ADMINISTRATION			TOTAL AMOUNT OF BILE COLLECTED IN THREE HALF HOUR PERIODS		PER CENT INCREASE
	3RD PER.	2ND PER.	1ST PER.	1ST PER.	2ND PER.	3RD PER.	BEFORE	AFTER	
	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	
1	1.1	1.1	0.9	1.6	1.3	0.9	3.1	3.8	22.6
2	0.7	0.7	0.7	1.8	2.2	2.2	2.1	6.2	195.2
3	0.7	0.6	0.6	2.0	2.7	2.7	1.9	7.4	298.5
4	0.7	0.7	0.5	2.1	1.9	1.8	1.9	5.8	205.4
5	0.4	0.4	0.4	1.0	0.5	0.5	1.2	2.0	66.7
6	1.4	1.2	0.8	1.4	1.8	1.5	3.4	4.7	38.2
7	1.5	1.1	0.6	2.4	1.3	0.9	3.2	4.6	43.8
8	2.1	1.9	1.6	3.2	3.4	3.1	5.6	9.7	73.2
Average							2.82	5.53	97.5

*150 mg. each of sodium taurocholate and sodium glycocholate plus 3 c.c. oleic acid.

experiments (100 per cent) showed an increase in bile output after the administration of the combined cholagogues. Three experiments showed yields of over 100 per cent: Exper. III with 289.5 per cent; Exper. IV with 205.4 per cent, and Exper. II with 195.2 per cent. Any one of these three figures exceeds the sum of the highest yields in Series I and II. The average of the total of the collections after the administration was 97.5 per cent higher than that of the same period before. This percentage is almost three times the sum of the two corresponding figures in Series I and II.

It is thus seen that the combination of the two cholagogues potentiate each other to a remarkable degree, whether judging from the number of experiments in each group in which a rise was registered, the percentage rise of the individual experiments in the three different series, or the ratio between the average of the total yield before and that after the administration. The potentiation is so striking as to merit the term synergism.

SUMMARY

In three series of experiments of eight experiments each, on the dog, in which the weight factor, the diet, the anesthetic level, and the dose of cholagogics given were kept constant, it was shown that choleresis caused by the combination of relatively small doses of oleic acid and of bile salts was much more marked than would be expected from the sum of the effects of the same doses of bile salts and oleic acid administered separately.

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THE RELATIVE EFFECTS OF DIATHERMY AND INFECTION ON THE PLASMA PROTEINS, PLASMA VISCOSITY AND SUSPENSION STABILITY OF THE BLOOD IN DOGS*

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THE beneficial effect of fever in the defense mechanism against infection was reviewed in a recent report by Reimann.¹ He also showed that an increase in plasma viscosity depending on an increase in the fibrinogen and globulin fractions of the blood enhanced specific agglutination and suggested that these protein changes which occur during infectious diseases play an important rôle in immune processes. In a previous study on lobar pneumonia² a prompt increase in the plasma globulin and fibrinogen fractions together with increased plasma viscosity was observed within a short time after the initial chill. It

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seemed possible that the high fever associated with this disease together with the severe toxemia may have been an important factor in inducing these blood protein changes.

Recent publications indicate an increasing use of artificial fever in the treatment of various diseases. The tendency toward the substitution of diathermy for the older and more difficultly controlled methods of inducing therapeutic pyrexia by infections or by the injection of foreign proteins necessitates inquiry as to how far increased body temperature resulting from diathermy duplicates the effects of fever due to infection or to the injection of foreign protein.

The object of the present study is to compare the relative effects of pyrexia produced by diathermy on the plasma proteins, the plasma viscosity and the suspension stability of the blood with the changes produced by experimental infectious fever of comparable duration. A search of the literature revealed that comparatively few observations have been made on the influence of diathermy and the ultra high frequency current on the blood proteins. Frisch and Starlinger³ and Hirsch⁴ observed slight changes in the blood proteins after local diathermy for short periods. Knudson and Schaible⁵ noted a slight increase in the total blood proteins of dogs heated by the ultra high frequency current.

METHODS

The first experiments were made on rabbits, but it was found that these animals succumb rapidly after being heated by diathermy. Dogs weighing from 10 to 16 kg. were then used. Nembutal (sodium-ethyl, 1-methyl-butyl barbiturate) in an initial dose of 30 to 40 mg. per kg. of body weight was given intravenously as a sedative. The effect of this drug lasted from four to eight hours. If subsequent restlessness occurred smaller supplementary doses were given subcutaneously. Nembutal anesthesia was also employed before intraperitoneal and intra-bronchial inoculations. This drug had but slight effect in reducing the normal temperature in an occasional dog. In one animal, a maximum reduction of 1° C. occurred in five hours.

During the diathermy treatment, the anesthetized animal was suspended in a hammock with its head supported in a sling. Excessive loss of body heat and moisture by respiration was prevented by the inhalation of steam rising from a basin of hot water on an electric plate under the dog's head, which was covered by a hood. Rectal temperature was measured at one-half to one hour intervals. Water was supplied through a stomach tube. The animals were weighed before and after treatment. The Fischer diathermy machine, type CDC with a spark gap oscillator of an approximate frequency of 1,000,000 cycles was used. Copper gauze electrodes 10 by 15 cm. sewed to cotton pads 1 cm. thick and soaked in a sodium chloride solution were fixed with bandages to the shaved sides of the dog. A current density of approximately 10 milliamperes per square centimeter of electrode area was employed to raise the dog's temperature to the desired degree. The amount of current necessary to maintain the temperature at a certain level varied in different animals.

In order to produce a severe infection with rapid onset which would be accompanied by a fever, similar in degree to the pyrexia evoked by diathermy,

peritonitis was caused by the intraperitoneal injection of 5 to 15 c.c. of a saline suspension of dog feces (5 gm. per 15 c.c. suspension). In two instances, 3 c.c. of an eighteen-hour blood broth culture of Type I pneumococcus in 16 per cent gelatin solution were injected intrabronchially in an attempt to produce a pneumonia. In one animal, 3 c.c. of a Type I broth culture of pneumococcus were injected intrapleurally, and another was injected intraperitoneally with 10 c.c. of a broth culture of *B. welchii*.

Twenty to 25 c.c. of blood for analysis were withdrawn from the jugular or leg veins with a minimum of stasis, and oxalated. In some instances in which the animals were moribund blood was obtained by cardiac puncture. Plasma protein fractions were determined by Berglund's⁶ modification of Howe's method using sodium sulphate as a salting-out agent. Nitrogen determinations were made by the micro-Kjeldahl technic. The sedimentation time of erythrocytes was determined in a manner similar to that described in a preceding paper.² The longer sedimentation time of normal dogs' blood as compared to that of human blood is possibly due in part to the lesser amount of blood protein and the greater number of erythrocytes in dogs' blood. The viscosity of the plasma, and in several instances the viscosity of the whole blood was measured with the Hess viscosimeter. Corpuscle and plasma volume determinations were made by centrifugation of blood in a tuberculin syringe at 2,000 revolutions per minute for one-half hour.

RESULTS

Effect of Pyrexia Produced by Diathermy on the Protein Fractions and Viscosity of the Plasma.—Eight dogs were heated with diathermy for variable periods of time. Pyrexia usually developed within one hour after starting diathermy. The duration of the fever was ten hours in three dogs, eight hours in two dogs and nine, eleven, and twelve hours in each of three dogs, respectively. The maximum temperature attained in the animals varied from 40.5° C. to 42.5° C. The four dogs heated to the highest temperatures died shortly after completion of the experiment. One dog heated to a maximum of 41° C. with an average temperature of 40.5° C. for ten hours, died about eighteen hours after completion of the experiment. The other three dogs heated for eight, ten, and twelve hours survived. When the desired temperature had been reached in several of the dogs, diathermy was discontinued and the temperature was maintained at a fairly constant high level by the inhalation of steam; for instance, after raising the temperature in one animal, diathermy was discontinued and the temperature was maintained between 41° C. and 41.3° C. for nine hours by steam inhalation. In several dogs, intermittent use of diathermy was sufficient to maintain pyrexia.

The effect of pyrexia produced by diathermy on the total plasma protein was variable (Table I). In two dogs (3 and 5) an increase in amount occurred within a few hours and in six dogs a decrease was noted. The total globulin fraction was decreased in five dogs and only slightly increased in three (3, 4, and 13). The amount of fibrinogen was decreased in four instances (2, 3, 12, 13) and slightly increased in four (1, 4, 5, and 6). The most constant changes in the

TABLE I
EFFECT OF DIATHERMY ON PLASMA PROTEINS

DOG	DURA- TION OF FEVER	RECTAL TEMPERATURE C.		TOTAL PLASMA PROTEIN	ALBU- MIN	GRAMS PER 100 C.C. PLASMA						FIBRIN- OGEN	EUGLOB- ULIN	PSEUDO-		ALBUMIN GLOB- ULIN RATIO	VISCOSITY OF PLASMA	ERYTH- ROCYTE VOLUME PER CENT	COMMENT
		MAXIMUM	AVERAGE			TOTAL GLOB- ULIN	PSEUDO- GLOB- ULIN I	PSEUDO- GLOB- ULIN II											
1	Before		38.8	5.92	3.13	2.79	0.20	0.80	1.23	0.56				1.12	1.50		Died several minutes after treatment		
2	10 hr.	42.5	41.6	5.66	3.51	2.15	0.32	0.38	0.78	0.68				1.63	1.60	63			
	Before		38.5	5.12	2.76	2.36	0.57	0.23	0.59	0.95				1.17	1.50		Died 30 minutes after treatment		
3	8 hr.	42.0	41.2	4.88	2.93	1.95	0.14	0.28	0.73	0.79				1.50	1.40				
	Before		38.9	4.68	2.60	2.08	0.37	0.14	0.70	0.87				1.25	1.60		Died 1 hr. after treat- ment		
4	11 hr.	41.8	41.2	5.02	2.53	2.49	0.33	0.30	0.60	0.75				1.01	1.60				
	Before		37.0	5.14	3.40	1.74	0.22	0.23	0.53	0.76				1.95		38	Survived		
5	12 hr.	40.5	40.3	4.98	3.24	1.74	0.15	0.24	0.91	0.45				1.86		47	18 hr. later. Electrode burn		
	Before		37.2	5.51	3.47	2.04	0.40	0.19	0.77	0.69				1.70					
6	10 hr.	41.0	40.4	5.08	3.09	1.99	0.35	0.28	0.77	0.59				1.55	1.50	47			
	Before		38.7	5.83	4.12	1.71	0.58	0.14	0.80	0.19				2.41	1.50	60	Died 18 hr. after treatment		
12	10 hr.	40.5	40.0	5.37	3.35	2.02	0.40	0.28	0.66	0.68				1.65	1.60	40	Survived		
	Before		38.5	5.02	3.13	1.89	0.52	0.28	0.47	0.63				1.65	1.60	42			
13	8 hr.	40.8	40.2	5.67	1.99	3.68	0.98	0.52	1.45	0.73				0.54		25	Survived		
	Before			5.47	2.23	3.24	0.13	0.52	1.59	1.01				0.68		35			
13	Before			5.28	2.09	3.19	0.92	0.28	1.31	0.68				0.65		25	Two days after di- athermy treatment		
				5.76	2.60	3.16	0.23	0.56	1.25	1.12				0.82	1.70	21	Six days after diath- erny treatment		
		41.2	40.5	5.23	2.98	2.25	0.78	0.19	0.83	0.46				1.32	1.55	33	Died several hours after treatment		
				5.25	2.93	2.32	0.56	0.24	0.84	0.68				1.26	1.55	51			

plasma protein fractions were a slight increase in amount of albumin and a corresponding decrease of total globulin. The viscosity of the plasma was usually unchanged.

Effect of Severe Rapidly Fatal Peritonitis on the Protein Fractions and Viscosity of the Plasma.—Six dogs were injected intraperitoneally with 5 to 15 c.c. of a saline suspension of dog feces. Four of the six dogs were moribund within twelve hours. Blood proteins were determined usually before and from eight to twelve hours after the fecal injection. Five of the six dogs developed fever about three hours after the fecal injection with maximum temperature between 40.6° C. and 42° C. One animal developed no fever and died in a condition of shock eight hours later.

The total plasma proteins were slightly increased (Table II) in four animals and slightly decreased in two during the first twenty-four hours. The fibrinogen was appreciably increased in two animals (9 and 10), decreased in one (14) and remained unchanged in three (4, 8 and 12) during twenty-four hours. In Dog 12, the total protein increased to 6.35 gm. and the fibrinogen to 0.82 gm. five days after infection. There was a slight increase in the albumin fraction in five dogs and a slight decrease in the total globulin fraction in three. The euglobulin and pseudoglobulin fractions were variable.

Only slight changes in the plasma viscosity were noted in these six animals. The viscosity of the whole blood in Dog 10 before and after infection was found to be 4.9 and 8.2, respectively. The increase was due apparently to the marked increase in red cell volume from 33 to 62 per cent.

Effect of Prolonged Infections on the Plasma Proteins and Viscosity of the Plasma.—Three dogs were used. A number of procedures were carried out on two of the animals. Dog 6 was injected intraperitoneally with 15 c.c. of a suspension of feces. This was followed by a fever of 40.4° C. The animal recovered rapidly and the next day the temperature was normal. Unfortunately the blood specimen taken before the intraperitoneal injection was accidentally discarded. However, twenty-four hours after the fecal injection the blood protein fractions were within normal limits as shown in Table III. Two days later or three days after the fecal injection, marked changes in the blood proteins were observed. The total plasma protein increased from 5.60 gm. per 100 c.c. to 8.51 gm., the fibrinogen from 0.54 gm. to 3.44 gm., the total globulin from 2.62 gm. to 5.48 gm. while the amount of albumin did not change. Ten cubic centimeters of a brain broth culture of *B. welchii* were then injected intraperitoneally, and twenty-four days later 3 c.c. Type I pneumococcus in 16 per cent gelatin solution were injected intrabronchially with no apparent effect on the animal. After fifty days the fibrinogen and total globulin were still increased, although reduced from the maximum figures observed three days after the fecal injection. The red cell volume in this dog varied from 22 per cent to 56 per cent, the higher values occurring during periods of fever. The viscosity of the plasma was increased from 1.70 to 1.90 during the period of high total globulin and fibrinogen.

In Dog 11, 3 c.c. of an eighteen-hour blood broth culture of Type I pneumococcus in a 16 per cent gelatin solution were injected intrabronchially in an attempt to produce pneumonia. Practically no fever developed and the next

TABLE II
EFFECT OF RAPIDLY FATAL PERITONITIS ON PLASMA PROTEINS

DOG	HOURS AFTER INTRAPERITO- NEAL FECAL INJECTION	MAXIMUM RECTAL TEMPERA- TURE C.	TOTAL PLASMA PROTEIN	ALBU- MIN	GRAMS PER 100 G.C. PLASMA										FIBRIN- OGEN	EUGLOB- ULIN	PSEUDO- GLOBU- ULIN I	PSEUDO- GLOBU- ULIN II	ALBUMIN } GLOBULIN }	RATIO	VISCOS- ITY OF PLASMA	ERYTH- ROCYTE VOLUME PER CENT	COMMENT
					TOTAL GLOBU- LIN																		
4	0	38.3	5.38	2.67	2.71	0.65	0.38	0.86	0.83	0.98	1.70	42	Died 3 days after intra- peritoneal injection										
	24	41.7	4.72	2.77	1.95	0.64	0.19	0.61	0.52					1.42	1.65	58							
8	0	38.5	4.99	2.60	2.39	0.58	0.23	0.70	0.87	1.08	1.70	35	No fever. Moribund										
	8	38.5	5.77	3.40	2.37	0.56	0.52	0.97	0.32					1.44	1.60	65							
9	0	38.5	5.41	3.12	2.29	0.44	0.24	1.05	0.57	1.36	1.60		Moribund										
	10	41.0	6.41			0.73	0.61																
10	0	38.2	5.46	2.93	2.53	0.40	0.28	0.94	0.91	1.15	1.60	33	Moribund										
	12	40.8	5.77	3.61	2.16	1.04	0.28	0.30	0.55					1.67	1.60	62							
12	0	39.2	5.76	2.60	3.16	0.23	0.56	1.25	1.12	0.82	1.70	21	Moribund										
	9	40.6	5.24	2.60	2.64	0.13	0.52	1.12	0.87					0.98	1.70	30							
14	24	39.8	5.48	2.46	3.02	0.23	0.33	1.42	1.04	0.81	1.90	21	Died										
	5 days	40.5	6.35	2.77	3.58	0.82	0.33	1.20	1.23					0.77	1.80	11							
		0	38.2	5.47	2.93	2.54	0.54	0.23	0.69	1.07	1.15	1.60		37									
		9	42.0	6.20	3.91	2.29	0.26	0.60	0.78	0.65	1.70	1.55		74	Died								

day the dog appeared well. At the end of four days, during which time the temperature was normal, the blood fractions showed only minor changes. Three cubic centimeters of Type I pneumococcus broth culture were then injected intrapleurally. One-half hour following the intrapleural injection, the animal developed a severe chill, but fever did not develop. Seven days after the intrapleural injection, marked changes in the blood proteins were observed with increase in the total plasma protein, the total globulin and fibrinogen. The fibrinogen especially increased, rising from 0.63 gm. to 1.50 gm. After a period of seven days, 5 c.c. of a fecal suspension were injected intraperitoneally, following which the animal developed a fever reaching 40.4° C. Twenty-four hours later the dog was moribund and died shortly after blood was taken for analysis. The albumin fraction was increased and the total globulin fraction decreased as in the preceding experiments in which peritonitis was produced. In spite of the total globulin decrease, the fibrinogen showed further increase to 1.63 gm.

Dog 15 was injected intraperitoneally with 15 c.c. of a fecal suspension. A maximum temperature of 41° C. was reached four hours later, but the next day the temperature returned to normal. The changes in the plasma proteins, erythrocyte volume and plasma viscosity (Table III) were similar to those described in Dogs 6 and 11. The total plasma protein and fibrinogen had decreased by the end of ten hours but two days later both were increased, especially the fibrinogen. On the other hand, the total albumin increased slightly and the total globulin diminished. The viscosity of the plasma was greatest at two days when the fibrinogen was at its height.

Effect of Pyrexia Produced by Diathermy and by Infection on the Sedimentation Time and Red Cell Volume Percentage.—A constant increase in the sedimentation time of erythrocytes was noted during fever whether it was produced by diathermy or by infection. A numerical value for the sedimentation time was not possible in many instances as the red cell volume did not settle to the arbitrary level used in our method. The increased suspension stability of the blood was probably accounted for by the increase in red cell volume percentage which many have shown to have a direct bearing on erythrocyte sedimentation. The erythrocyte volume was increased 5 to 55 per cent above the normal during diathermy heating and 43 to 100 per cent above the normal during fever of infectious origin. The sedimentation time approached normal in the animals that survived diathermy heating when the red cell volume percentage dropped to its former level. In the animals with prolonged infections, the sedimentation time was shortened when the cell volume was normal and the globulin and fibrinogen fractions increased.

DISCUSSION

In drawing conclusions from our findings, attention must be directed to the fact that strictly comparable conditions could not be obtained in the three sets of experiments because of the inability of the animals to survive the desired degree and duration of pyrexia produced by diathermy. Therefore no measurements have been made of the protein fractions of the dogs heated with diathermy after the length of time that had to elapse before a significant change had taken place

in the dogs which had infectious fever. The marked increase in red cell volume percentage during fever with probable decrease in total blood and plasma volume also added complicating factors.

In Table IV are recorded the mean values of rectal temperature, plasma viscosity, total plasma protein, and the various protein fractions during the control periods and the three experimental periods, together with the probable errors

TABLE IV

MEAN VALUES OF RECTAL TEMPERATURE, BLOOD VISCOSITY, TOTAL PLASMA PROTEIN, FIBRINOGEN, TOTAL GLOBULIN AND ALBUMIN DURING CONTROL PERIODS, DURING DIATHERMY AND IN THE COURSE OF RAPIDLY FATAL AND PROLONGED INFECTIONS

	NO. OF OBS.	CONTROL	NO. OF OBS.	DI- ATHERMY	NO. OF OBS.	RAPIDLY FATAL INFECTIONS	NO. OF OBS.	PROLONGED INFECTIONS
Rectal tempera- ture	16	38.4 ± 0.11	8	40.7 ± 0.15	8	40.6 ± 0.09	11	39.2 ± 0.20
Plasma viscos- ity	13	1.62 ± 0.02	6	1.56 ± 0.03	7	1.69 ± 0.03	10	1.83 ± 0.02
Total protein gm./100 c.c.	16	5.42 ± 0.06	11	5.33 ± 0.07	8	5.74 ± 0.05	11	6.37 ± 0.19
Fibrinogen gm./100 c.c.	16	0.50 ± 0.04	11	0.39 ± 0.05	8	0.55 ± 0.08	11	1.25 ± 0.18
Total globulin gm./100 c.c.	16	2.53 ± 0.09	11	2.35 ± 0.12	7	2.57 ± 0.16	11	3.22 ± 0.19
Albumin gm./100 c.c.	16	2.86 ± 0.06	11	2.98 ± 0.12	7	3.07 ± 0.15	11	3.15 ± 0.11

of the means. In Table V are the ratios of the differences of the means to their probable errors computed for the control periods (a) and of the three experimental periods (b). According to statistical standards, when this ratio is greater than 3 the difference between the means becomes of significance, when approximately 3 it is of doubtful significance, and of no statistical significance when less than 3.

Increase of total protein was apparently not dependent upon elevated temperature, since the group subjected to diathermy, in which temperature was definitely raised, showed no rise in plasma proteins and in the group with prolonged infection, the proteins rose while the rise in temperature was only slight. In the case of the rapidly fatal infection, with high fever, the total protein was

TABLE V

Mb - Ma

$$\sqrt{PE_{Mb}^2 + PE_{Ma}^2}$$

	CONTROL (A) DIATHERMY (B)	CONTROL (A) RAPIDLY FATAL IN- FECTION (B)	CONTROL (A) PROLONGED INFECTION (B)
Rectal temperature	+ 12.63	+ 16.05	+ 3.50
Plasma viscosity	- 1.93	+ 1.84	+ 7.76
Total protein	- 0.98	+ 4.26	+ 4.85
Fibrinogen	- 1.81	+ 0.21	+ 3.69
Total globulin	- 1.20	- 0.11	+ 3.00
Albumin	+ 0.88	+ 1.53	+ 2.64

higher than normal. It seems then that elevation of the total protein level in these experiments was associated with infection regardless of type and not with pyrexia.

The increase in total protein was not associated invariably with increase of either fibrinogen, albumin, or total globulin. In the case of the rapidly fatal infection the reaction of each group was so variable that none showed a significant rise, whereas, in the prolonged infection, the ratios of the differences of the means to their probable errors recorded in Table V indicate that the rise in value of the fibrinogen is of definite statistical significance, and the increases in both albumin and globulin approach significant ratios. These findings are in accord with those of Hurwitz and Meyer⁷ and others who showed definite increases in the globulin fraction during various immunization processes in which fever was not a prominent feature.

Plasma viscosity was moderately increased only in the group with prolonged infection. Its rise was, therefore, not associated with a high fever but with a high fibrinogen and total globulin content of the blood as seen also from inspection of individual cases in Table III.

The concentration of the blood with marked increase in the red cell volume percentage following artificial heating of dogs has been observed by others.⁵ The total blood volume was found to be definitely decreased during heating with the ultra high frequency current⁵ and with diathermy.⁸ That these changes cannot be accounted for on the basis of general body dehydration was indicated by our experiments in which loss of body weight was prevented by the giving of water by stomach tube and by the inhalation of water vapor. Some of the animals gained slightly in weight during diathermy heating due to excessive water intake. It would appear that a redistribution of the plasma with a shift from the blood to the tissue spaces had occurred.

Interpretation of the sedimentation times of erythrocytes in regard to their correlation with the plasma protein changes was difficult of analysis because of the marked increase in the red cell volume during fever. The increase in the suspension stability of the blood during fever in dogs appeared to be directly proportional to the percentage increase of erythrocyte volume. Others⁹ have also found an increase in suspension stability during diathermy heating, while Bernet,¹⁰ on heating rabbits for shorter periods, found no change.

SUMMARY

1. Slight and variable changes were produced in the plasma protein fractions of dogs by pyrexia due to diathermy over periods of from eight to twelve hours. In general, the changes were in the direction of a reduction, but in no case did this reduction attain a statistically significant value.

2. During infectious fevers of short duration the total protein was definitely elevated, but the shifts in the individual fractions were so variable that in no single fraction was the rise significant.

3. In certain prolonged infections even without marked rise of body temperature the total protein was definitely elevated. Albumin, total globulin and fibrinogen were all increased in amount, the rise in fibrinogen being most marked.

4. The only significant change in plasma viscosity was in the group with prolonged infection. Here the viscosity was moderately increased and was probably dependent on the high fibrinogen and globulin content of the plasma.

5. Correlation of changes in the erythrocyte sedimentation time with fractional plasma protein variations in these experiments were difficult of analysis because of the marked increase of the erythrocyte volume in dogs during fever.

6. Although conditions of the three sets of experiments were necessarily not identical, it is suggested from our data that fever alone is not an important factor in evoking the plasma protein changes usually observed in infectious diseases.

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DESTRUCTION OF PYOGENIC BACTERIA IN THE ALIMENTARY TRACT OF SURGICAL MAGGOTS IMPLANTED IN INFECTED WOUNDS*

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IN THEIR natural surroundings, blowfly larvae feed upon dead and decaying animal tissue. When used clinically, that is, when bred aseptically and implanted as sterile maggots in infected wounds, they function largely to remove dead tissue and pus from the wound. At the same time the maggots take up large numbers of bacteria from the wound and by destroying them aid in reducing the infection.¹⁴

The present investigation was made to determine the relative abundance and viability of the bacteria in the successive regions of the alimentary tract.

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This was done by means of dissections and bacteriologic cultures. The species used was *Lucilia sericata*. A description of the technic and apparatus, particularly that used in the aseptic dissections, is included, as descriptions of special methods are sometimes of interest.

DISSECTIONS AND CULTURES

Description of the Digestive Tract.—The tract is tubular, highly convoluted, and very long, being 90 to 95 mm. in length, or six to seven times longer than the full-grown maggot. To the short and very narrow esophagus is attached a sac for holding food in reserve. This lies along the back and when containing food is visible through the integument. The stomach is unusually long, generally 60 to 65 mm. and comprising two-thirds of the entire tract. It is divided histologically and functionally into three sections, the fore-, mid-, and hind-stomach. It extends from the enlargement at its junction with the esophagus posteriorly to the malpighian tubes. The intestine is about 30 mm. long and extends from the malpighian tubes to the anus. Greater details may be found in descriptions by Hobson,⁹ Lowne,¹¹ and Snodgrass.¹⁶

Technic of Dissection.—The maggots were reared under the usual sterile conditions,^{4, 5, 6, 12, 13, 17} to about $\frac{1}{4}$ to 6 mm. in length, the size suitable for introduction into wounds. The sterile maggots were implanted in osteomyelitis wounds and allowed to feed. In twenty-four to forty-eight hours they were large enough to dissect and meanwhile the food had passed freely through the tract. The maggots were then removed and killed by momentary dipping in boiling water. Their external surface was sterilized by immersion for fifteen minutes in mercuric chloride disinfectant,^{4, 13, 18} and washed in two changes of sterile saline. The sterility of the external surface was confirmed with cultures which showed no growth. The sterility of the alimentary tract had likewise been confirmed before the maggots were implanted in the wounds. The maggots were ready for dissection within thirty minutes of removal from the wound.

Dissections were made under a binocular microscope. Each specimen was opened under sterile saline in a Petri dish, and aseptic technic was used throughout. In opening the maggots, a pair of fine dissecting scissors, with the cutting edges at an angle with the handles, was found to be the most satisfactory. The extreme points of the scissors were taken off with an abrasive to prevent piercing the intestinal tract. Forceps with fine curved points were used in making the dissections.

When uncoiling the alimentary tract and disconnecting it from the branching tracheas, especial care was taken to avoid rupture of the tract and consequent contamination of the saline. Notwithstanding this, rupture occurred frequently and the specimens had to be discarded. As an additional precaution against contamination through any possible rupture, dissections were begun upon the intestine, which always had the least infection, and the fore-stomach was removed last. Approximately 20 mm. of each portion was removed. The sections were cut off above the saline with a red-hot platinum wire in order to sear the remaining ends of the tract and thus prevent contamination of the saline.

Cultures.—In the series comprising Table I, the cultures were made by macerating and smearing the various sections of the tract upon the surface of blood agar plates. Those in Table II were macerated in nutrient broth tubes. A control culture of the saline in the dissecting dish was made in each case after the maggot had been opened but before dissection of the tract.

RESULTS

Dissections were made successfully of 51 individuals. Because of the decisive nature of the results and also because of the difficulty in dissecting without rupture the delicate and numerous convolutions of the tract, the experiments were limited to this number.

In cultures from the intestine, no bacterial growth at all was observed in 26 of the specimens listed in Table I. In the two remaining specimens a slight growth was found but at the same time the saline also showed contamination before dissection was begun.

TABLE I
CULTURES ON BLOOD AGAR

SPECIMEN	CONTROL (SALINE BEFORE DISSECTION)	INTESTINE	HIND- STOMACH	FORE- STOMACH
1	—	—	—	++*
2	—	—	+	+++
3	—	—	—	+++
4	—	—	+	+++
5	—	—	—	++
6	—	—	—	+
7	—	—	+	+
8	—	—	—	++
9	—	—	—	++
10	—	—	—	++
11	+	+	+	++++
12	—	—	—	+++
13	—	—	+	++++
14	—	—	—	+++
15	—	—	—	+++
16	+	+	—	++++
17	—	—	+	++++
18	—	—	—	+++
19	—	—	+	+++
20	—	—	—	++
21	—	—	+	+++
22	—	—	—	+++
23	—	—	+	++
24	—	—	—	++
25	—	—	—	++
26	—	—	+	+++
27	—	—	+	+++
28	—	—	—	++++

*The number of plus signs indicates the relative density of growth. One indicates ten or less colonies, and four represent one hundred or more colonies. Sections of tract were only approximately of equal length.

The hind-stomach was also found to be sterile in 17 specimens. The remaining 11, among which were the two cases above mentioned, showed slight growth.

In every case bacterial growth was obtained in cultures from the fore-stomach; and in all but two instances the growth was abundant. The relative degree of growth is indicated in all cases by the number of plus signs, from one to four.

TABLE II
CULTURES IN NUTRIENT BROTH

SPECIMEN	CONTROL (SALINE BEFORE DISSECTION)	INTESTINE	HIND- STOMACH	FORE- STOMACH
1	-	-	-	+
2	-	-	-	+
3	-	-	-	+
4	-	-	-	+
5	-	-	-	+
6	-	-	-	+
7	-	-	-	+
8	-	-	-	+
9	-	-	-	+
10	-	-	+	+
11	-	-	+	+
12	-	-	-	+
13	-	-	-	+
14	-	-	-	+
15	-	-	-	+
16	-	-	-	+
17	-	-	-	+
18	-	-	-	+
19	-	-	-	+
20	-	-	+	+
21	-	-	-	+
22	-	-	-	+
23	-	-	-	+

*Degree of growth in this medium cannot be shown because a general clouding of the broth takes place.

A more rigid test was made in the second series when dissections were cultured in nutrient broth. In this medium the presence of even minute numbers of bacteria becomes evident in the clouding of the broth.

The sterility of the intestine and the presence of bacteria in the fore-stomach, indicated in the first series, were confirmed in every instance. The results are shown in Table II.

DISCUSSION

Disappearance of Bacteria in the Stomach.—It has been shown that, in their course through the alimentary tract of the maggot, bacteria become reduced in numbers and finally disappear. It is evident, from the sterility of the intestine, that the bacteria are destroyed in the stomach. It is also apparent that this action takes place principally in the posterior portion of the stomach. The destruction of bacteria in this way is probably a factor in the general disinfection of the wound under the maggot treatment, as noted by Baer³ and confirmed by others.

The species of bacteria encountered in the experiments were a betahemolytic streptococcus and a hemolytic type of *Staphylococcus aureus*. Cases were selected in which no organisms of the proteus type were present. This was because of the difficulty of making quantitative estimates of the numbers of bacteria

present with those spreading varieties. Hobson¹⁰ has made an examination of the general bacterial content of the alimentary tract of nonsterile maggots which had fed under normal conditions.

Results somewhat similar to those of the present investigation were reported by Duncan,⁷ who referred to some experiments of his own and of others in which destruction of certain species of bacteria was found to take place in the alimentary canal of flies.

Bacot,^{1, 2} Glaser,⁵ and Smith-Graham,¹⁵ however, using flies of the family Muscidae, found that bacteria ingested by the larvae with their food persisted through metamorphosis and appeared in the intestine of the adult flies. This may indicate that the larvae of those species are unable to destroy all bacteria within the alimentary tract. It seems possible, however, that the adult tissues may have become inoculated from the bacteria which were still present in the fore-stomach of the maggot at the time of pupation. By means of a special technique, Wollman¹⁰ was able to rear adult flies free from infection.

The disappearance of the bacteria between the fore-stomach and the intestine, in the region of greatest activity of proteolytic enzymes, according to Hobson,⁹ indicates that the bacteria are destroyed by digestion.

SUMMARY

A study has been made of the relative abundance and viability of bacteria in the various sections of the alimentary tract of surgical maggots used to hasten healing of infected wounds. The investigation was made by means of dissections and bacteriologic cultures.

The results are given in tabular form and show that large numbers of the bacteria taken in with the food were destroyed in passing through the long, tubular stomach of the maggot. Complete destruction of any remaining organisms occurred in the intestine, for no viable bacteria were found in any cultures of the intestine.

The results are discussed in connection with the work of others of a somewhat similar nature.

A description is given of the method used in making the aseptic dissections.

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BENIGN ADENOMA OF THE SWEAT GLANDS*

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PINGAUD¹ in 1877 reported a sudoriparous adenoma of the scalp. His gross and microscopic study was given in detail. He gave the impression that it was a small elastic fibromatous mass which was smooth and shining. There was no definite line of demarcation between the tumor and the skin. The histologic examination showed that the skin covering the tumor was intact and was separated from the neoplasm by a layer of normal connective tissue. It was lobulated like a gland. Each lobule contained a small amount of fibrous stroma composed of connective tissue. The stroma circumscribed an elongated or rounded network which contained epithelial cells. There were no epithelial pearls. In 1881 Sangster² reported a papillary tumor of the scalp. The gross and histologic study gave a picture similar to that of Pingaud. Mulert³ in 1897 called this type of tumor an endothelioma. Aimes and Paulet⁴ in 1921 gave a description of massive sudoriparous adenoma of the scalp which was very similar to that of Pingaud. Dupont and Delarue⁵ in 1928 described a sudoriparous tumor, but it was of the type which communicated with the exterior.

This case is presented because so few have been described in the literature, and because it has been designated by various authors as "benign epithelioma," "adenoma of the skin," "endothelioma," and "papillary tumor" of the scalp. This tumor was removed from over the right parietal bone. The growth first appeared about ten years previous in a man sixty-two years old, and had regularly and slowly increased in size without causing any

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pain. The specimen weighed 37 gm. and measured 6 by 4 by 3 cm. It was oval with a short stalk-like attachment that had been resected and measured 2 by 2 cm. at this lower stalk attachment margin. There were a few grayish hairs extending along the cut margin. The rest of the surface was hairless, white, and smooth. The tumor was well-encapsulated and numerous cut surfaces were white and homogenous. There was a general firmness with consid-

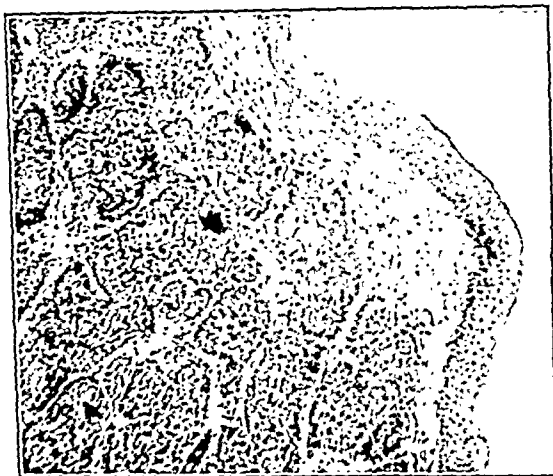


Fig. 1.—Sixteen mm. obj., showing lobulation with epidermis and dermis.



Fig. 2.—Four mm. obj. Cord-like arrangement of epithelial cells. Palisaded cells of periphery of lobules.

erable elasticity. Microscopic examination showed an epidermis with rather thin stratum corneum, a poorly defined stratum granulosum and a Malpighian layer with a lack of tendency to form definite intercellular bridges. The dermis consisted chiefly of connective tissue with sparse blood vessels. The tumor proper had well-formed cord-like arrangement of epithelial cells and some lobulation simulating glands surrounded by connective tissue. These lobulated groups were filled with cells, the major portion of which were flat and

oval; however, round and spindle-shaped types could be seen. A few contained elongated nuclei surrounded by mitochondria. Those in the periphery were palisaded with connective tissue as a base. The central cells in the large lobules were arranged indiscriminately.

The possibilities as to the origin of this tumor are: (1) the basal layer of the epidermis; (2) the outer root sheath of the hair follicle; (3) the ducts and bodies of the sweat glands; and (4) the sebaceous glands. If we consider the views of Krompecher⁶ then the absence of fibrillation of the constituent cells and the lack of tendency to form definite intercellular bridges rules the first two possibilities out. This leaves for consideration the sweat glands and the sebaceous glands. At some places in the tumor there is a tendency to form a two-layered epithelium surrounding the central cavity, the outer being of the columnar sort and the inner layer showing a tendency toward the squamous type. The presence of the colloid-like masses in the epithelial cells here and

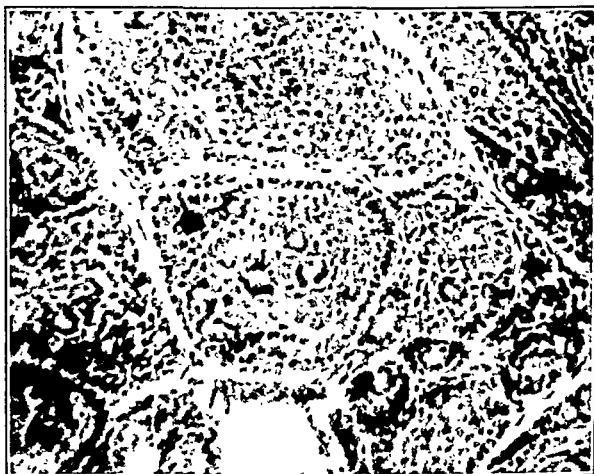


Fig. 3.—Four mm. obj. The colloid-like masses which have resisted solvents and were not differentiated by fat stain.

in the lumina gives the tumor a general resemblance to those tumors described^{7, 8, 9} as sebaceous gland tumors. These colloid-like masses, however, are obviously not of fatty nature because they resisted not only the solvents used in embedding but were ruled out by fat-staining methods and subsequent treatment of the section.

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7018 CREIGIER AVENUE

INTRACUTANEOUS REACTIONS INDUCED IN GUINEA PIGS INOCULATED WITH *B. ABORTUS**

A PRELIMINARY REPORT

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THE diagnosis of undulant fever is particularly difficult because of the marked variation in clinical manifestations, and, consequently, may be quite dependent upon laboratory tests. As microorganisms of the *abortus-melitensis* group are demonstrated in the blood stream with difficulty, cultural examinations are of limited value. At present, the agglutination test, which is in general use, is considered relatively specific, although the reactions obtained may, in some cases, be due to a past infection. On the other hand, Montagnani,¹ Burnet,² Tramontano,³ Carpenter and Boak,⁴ Giordano and Sensenich,⁵ and Gilbert and Dacey⁶ have reported failure to obtain agglutination in sera from patients having undulant fever. In view of these findings, an intracutaneous test might furnish valuable information.

Burnet,⁷ Trenti,⁸ and Dubois and Sollier⁹ obtained specific reactions with broth filtrates, while Montagnani,¹ Fornaca and Bua-Fazio¹⁰ and Giordano,¹¹ on the other hand, failed to do so. Fornaca and Bua-Fazio,¹⁰ Mitra,¹² Canale,¹³ de Fermo,¹⁴ Giordano,¹¹ Simpson and Frazier,¹⁵ and Levin¹⁶ report specific reactions with heat-killed suspensions of the microorganisms. Schoenholtz and Meyer¹⁷ found that "cutaneous hypersensitiveness in *abortus* infected guinea pigs may be demonstrated by testing the infected animal intracutaneously with filtered cell solution of *B. abortus*. The substance precipitated by acetic acid in the cold at P_H 3.5 to 4.0 elicits allergic reactions as effectively as the original solution." In 1931, Leavell and Amoss¹⁸ published a report of a study of the endermic reaction in *Brucella* infections in which various extracts and suspensions including a so-called "specific soluble substance" were tested. They found none which they considered definitely specific. Human subjects were used for the investigation. A species of animal not subject, under normal conditions, to infections with members of the *abortus-melitensis* group may be found more suitable for purposes of control than persons who may have had undulant fever which was undiagnosed or an infection which was subclinical in nature. One would judge

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from the information available in the literature that allergic reactions may be demonstrated for a number of years following recovery from such an infection.

The results of a preliminary study of this problem undertaken during 1931 may be of interest. A number of the authors mentioned have reported that the reactions with bacterial suspensions and with protein fractions have been very severe, in certain individuals, those with the former often being accompanied by more or less serious general symptoms. In our experience, the intracutaneous inoculation of heat-killed suspension has elicited a reaction in 18 of 22 guinea pigs infected with microorganisms of the abortus-melitensis group. The reactions have been very severe and characterized by congestion, edema, and marked necrosis. Eighty-eight presumably normal guinea pigs and 27 which had been inoculated with material submitted for the routine examination for tubercle bacilli were also tested. Four, or 3 per cent, showed slight nonspecific reactions.

"Abortin" prepared according to the method of Seyfarth¹⁹ not only failed to elicit reactions in certain guinea pigs known to have been infected with *B. abortus*, but induced reactions in some apparently normal animals. Tests were also performed with a concentrated and purified "abortin" prepared by precipitation of the protein with acetone, according to the method of Wadsworth and Quigley²⁰ for the concentration and purification of the toxin of the *Streptococcus hemolyticus*. Definite reactions were obtained in 8 of 10 guinea pigs known to have been infected with microorganisms of the abortus-melitensis group. Another guinea pig inoculated with the same culture gave no reaction, but since this animal died of intercurrent infection and the microorganism with which it had been inoculated was not recovered, the significance of the result is indeterminate. No reactions were obtained following the first inoculation in eleven apparently normal animals. Nonspecific reactions, possibly due to sensitization from the first series of tests, were observed in several of these guinea pigs upon reinoculation, one week after the first skin tests. With this material, as with the bacterial suspensions, the reactions were very severe, corresponding in this respect to the results reported by other workers. One guinea pig infected with microorganisms of the abortus-melitensis group reacted to the concentrated uninoculated broth used for purposes of control, as well as to the "abortin." The sensitivity of certain animals to protein derived from the medium has thus been demonstrated and necessitates careful control of all tests.

When this work was undertaken early in 1931, the study of intracutaneous tests in infections with microorganisms of the abortus-melitensis group had been restricted to bacterial suspensions, to culture filtrates, or to protein fractions of the bacteria. It therefore seemed important at that time to determine the activity of the carbohydrate fractions in an effort to find a less toxic antigen. Since the work of Heidelberger and Avery²¹ with the pneumococcus, specific soluble substances have been obtained from many other pathogenic microorganisms, such as the tubercle bacillus,²² the Friedländer bacillus,²³ the streptococcus,^{24, 25} *B. dysenteriae*,²⁵ *B. mallei*,²⁵ and the gonococcus.²⁶

A carbohydrate fraction was prepared by a modification of the method described by Wadsworth and Brown.²⁷

Seventy-two-hour cultures of a recently isolated strain of bovine origin (32219), grown on liver infusion agar, were killed by heating at from 60° to 65°

C. for thirty minutes. The growth was then suspended in 0.85 per cent salt solution. Two methods were used to disintegrate the cells. In one preparation, the suspension was made in 0.01 normal sodium hydroxide and boiled for one and one-half hours. The other was centrifugalized and the sediment resuspended in a very small amount of 0.85 per cent salt solution containing 0.2 per cent formalin. The latter was subjected to repeated freezing and thawing. In each case, after centrifugalization, the supernatant fluid was acidified with acetic acid to remove the protein and the carbohydrate substance was precipitated from solution with 95 per cent alcohol. The precipitate was dissolved in 0.85 per cent salt solution, acidified with acetic acid, and concentrated by boiling, any precipitate was removed, and the carbohydrate substance was reprecipitated by alcohol. This process was repeated until the acid coagulable proteins had been removed by acetic acid and the heat coagulable proteins by boiling. The final precipitate was washed with acetone and ether, and dried over calcium chloride in a vacuum desiccator. A protein reaction was not obtained by the biuret and xanthoproteic acid tests, and the Molisch test indicated the presence of carbohydrate. The amount of nitrogen in two preparations varied from 0.45 per cent to 0.56 per cent and the ash, from 12.24 per cent to 28.37 per cent.

Precipitating properties were demonstrated with *B. abortus* antiserum. Complement was fixed in the presence of immune serum. Intracutaneous inoculation of one of these preparations induced macular reactions, with no evidence of necrosis or sloughing in four guinea pigs infected with microorganisms of the *abortus-melitensis* group.

The results suggest that a carbohydrate substance extracted from microorganisms of the *abortus-melitensis* group may give reactions of a much milder character than those obtained with the whole microorganisms, or with a protein extract. Material which can be successfully used in skin tests on persons should give characteristic and specific reactions that are not very severe. A preparation of the protein and cleavage products, concentrated by the method of Wadsworth and Quigley,²⁰ might be entirely satisfactory for use in tests on animals, while the carbohydrate fraction, if the lack of toxicity which was observed is confirmed, would apparently lend itself more readily to a test for evidence of undulant fever in man.

Future work should be directed, first to the further purification of the carbohydrate substances, and, second, to a more extensive study of the intracutaneous reactions induced by those substances on animals and also on the human subject with a view to distinguishing the reactions of diagnostic significance; especially since carbohydrates which differ in their antigenic activity have been obtained from cultures of the pneumococcus.²⁷

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THE SPECTROPHOTOMETRIC INVESTIGATION AND DETERMINATION OF BILIRUBIN*

WITH A CONSIDERATION OF OXYHEMOGLOBIN AND ITS EFFECT ON THE
ESTIMATION OF BILIRUBIN

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INTRODUCTION

VISUAL spectrophotometry has been employed in a number of investigations on bilirubin. In 1925 and 1926, Mann, Sheard, Bollman and Baldes¹³⁻¹⁷ obtained extensive spectrophotometric data bearing on the question of the sites of formation of bilirubin, the relative amounts of bilirubin formed at the different sites, and the formation of the pigment from hemoglobin. In 1926 and 1927, Bollman, Sheard and Mann investigated the absorption of bile pigment from the intestine² and the bilirubinemia following extirpation of the gall-bladder and ligation of the common bile duct.³ Spectrophotometric comparisons of the nature and amount of bile pigment found in the blood serum under normal and pathologic conditions were made by Magath and Sheard¹² in 1927. The effect of intravenous injections of chlorophyll on the rate of formation of bilirubin was investigated by Bollman, Sheard and Mann.⁴ In these researches the spectrophotometric data were obtained by means of a Keuffel and Esser "color analyzer," which allows transmissions to be determined visually in a spectral range extending approximately to wave length 430 m μ (millimicrons).

Various data and conclusions concerning the spectrophotometry of bilirubin have been published by Sheard, Baldes, Mann and Bollman.¹⁹ This work will be referred to later. Sheard, Mann and Bollman²⁰ have made spectrophotometric comparisons of purified bilirubin and the yellow pigment (bilirubin) found in bile and in blood serum and plasma. In both of these investigations fading of the pigment is demonstrated, the effects of hemolysis and turbidity are described and discussed, and the laws of Lambert and of Beer are shown to be applicable to solutions of the pigment. These investigations have amply demonstrated the sensitivity, accuracy and practicability of the spectrophotometric method of investigating bilirubin.

In this paper we shall present and discuss the applications of spectrophotometry to investigations on the bilirubin in the blood and in other body fluids. In addition we shall present some observations and experimental results concerning spectrophotometric determinations of oxyhemoglobin and the

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effects of the presence of small amounts of this pigment on the quantitative determination of bilirubin.

The experimental data on which this article is based were obtained during the course of various investigations on bile pigment, concerning which a report will be made later. Our spectrophotometric observations are an extension of those made by Sheard and coworkers, to which reference has been made.

SPECTROPHOTOMETRIC THEORY AND ITS APPLICATIONS

To simplify the discussion we first review briefly some important principles of spectrophotometry and discuss their applications.

Consider a beam of light passing through a layer of solution of a substance X which is under investigation. Let I represent the intensity of the transmitted beam and I_0 the intensity which the beam would have had if it had been absorbed (in part) only by the solvent. Then the fractional part transmitted by X alone is $\frac{I}{I_0}$. Call this T , the transmission of X. Let C , L , ϵ and A represent the concentration, the thickness, the extinction coefficient (or absorption coefficient) and the absorption ratio, respectively. By definition, ϵ is the reciprocal of that thickness for which $T = 0.1$, while $A = \frac{C}{\epsilon}$, a constant.

It follows from Bouguer's law (commonly called Lambert's law) that

$$C = -\frac{A}{L} \log_{10} T. \quad (1)$$

If the value of A is known, this relation gives the concentration C upon substituting measured values of L and T . The expression $-\log_{10} T$ represents the optical density of X when measured in thickness L and concentration C .

It is easily shown from Equation (1) that for any one substance the ratio of the optical densities corresponding to any two wave lengths in the spectrum is a constant, regardless of the value of C or of L . Therefore, since no two substances have exactly the same transmission curve, a substance can be identified by showing that at various wave lengths the optical densities have the relative values known to be correct for that substance. Or, conversely, the entire transmission (or absorption) curve of a substance in solution can be calculated from the known values of the density ratios for the substance, if we know the value of T at some one wave length or can find it from the value of A and Equation (1).

In order that spectrophotometric observations on a solution may reveal only the absorption due to the dissolved substance, the absorption in the solvent, if not negligible, usually is balanced out by filling the comparison cell with the solvent. In physiologic fluids there are, in general, other substances in solution (or in colloidal suspension) besides the substance under investigation. All such substances absorb radiation; and since in many cases the absorption is not negligible, it is best, if possible, to balance it out by using as comparison fluid the identical physiological fluid, similarly diluted, with only the substance removed of which the transmission is to be measured.* If the substance cannot

*We usually neglect the error due to absorption in that part of the fluid which, in the comparison cell, occupies the space occupied in the other cell by the substance under investigation. In most cases the error is small.

be removed without altering the physical or chemical properties of the fluid, some fluid, such as distilled water, having negligible absorption, may be used as comparison substance. Correction must then be made for any appreciable absorption due to each of the other substances in the physiological fluid. The concentration of each substance must be found by some independent method and the corresponding transmission at each wave length computed by means of Equation (1). The transmission of the substance under investigation may then be found at any given wave length by dividing the observed transmission by the product of the transmissions of the other substances. In investigating bile pigment we have used this method in correcting for the absorption due to traces of oxyhemoglobin.

APPARATUS AND EXPERIMENTAL METHOD

The spectrophotometric observations were made by means of a quartz spectrograph and Hilger rotating sector photometer, with an under-water spark between tungsten terminals as source of radiation. These instruments were assembled and used in the usual manner, following essentially the method first described by Howe.⁹ The electrical circuits, quartz cells and container for the under-water spark have been described elsewhere.⁷

While the quartz spectrograph is to be preferred in most investigations because of its high dependability, our discussion of principles and methods will be understood to be applicable to the spectrophotometric investigation of bilirubin regardless of the kind of apparatus employed in finding the spectral transmission or absorption.

INTERFERENCE OF OXYHEMOGLOBIN WITH SPECTROPHOTOMETRIC OBSERVATIONS ON BILIRUBIN

It was found by spectroscopic examination that the majority of blood serums, however carefully prepared, contain traces of hemoglobin in the form of oxyhemoglobin, sufficient in amount to affect the absorption spectrum appreciably, even though the unaided eye may be able to detect little or none of the characteristic red color. No method is known whereby bilirubin alone can be removed from blood serum with the assurance that the serum will remain unchanged chemically or physically. This fact precludes the possibility of balancing out the absorption due to oxyhemoglobin (and other substances) by using the bilirubin-free serum as comparison fluid. Therefore in spectrophotometric measurements on bilirubin, corrections must be made for the radiation absorbed by oxyhemoglobin. The importance of this has been pointed out by Mann, Sheard, Bollman and Baldes¹⁴ in connection with visual spectrophotometric observations to determine the site of formation of bilirubin. Oxyhemoglobin has a very strong absorption band at the short-wave end of the visible spectrum, in the region covered by the most prominent and characteristic absorption band of bilirubin. The maximal optical density in the latter band is roughly twelve times as great as in the former. But we have found that the concentration of oxyhemoglobin in a serum which appears to the unaided eye to be free from red color actually may be much greater than the concentration of bilirubin; so that

the absorption in the oxyhemoglobin may be comparable to that in the bilirubin, in spite of the greater power of absorption of the latter.

To test the visibility of traces of oxyhemoglobin, various concentrations in water were prepared and the color noted when viewed against an illuminated white background. Thicknesses of solution of 1.5 and 9.0 cm. were examined, making direct comparisons with pure water. Data for the higher dilutions are given in Table I. Visual detection of oxyhemoglobin in blood serum is much

TABLE I
VISUAL APPEARANCE OF AQUEOUS SOLUTIONS OF OXYHEMOGLOBIN

HEMOGLOBIN, CONCENTRATION IN MG. PER C.C.	COLOR, VISUALLY OBSERVED THROUGH 1.5 CM. DEPTH	COLOR, VISUALLY OBSERVED THROUGH 9.0 CM. DEPTH
0.036	Not detectable, except when compared with pure water	Very faint pink, but noticeable
0.024	Not easily detected, even when compared with pure water	Hardly detectable, except when compared with pure water
0.007	Not detectable	Not detectable.

more difficult than in water, since the color of the serum tends to mask that of the oxyhemoglobin. We have found by spectroscopic and spectrophotometric measurements that amounts of from 0.05 to 0.10 mg. per c.c. frequently escaped detection by the unaided eye; while in one case a serum containing approximately 0.2 mg. of hemoglobin per c.c., or about 100 times the normal bilirubin content, appeared free from hemoglobin, the color being masked by that due to a high concentration of bilirubin. These concentrations of hemoglobin are easily high enough to modify the absorption spectrum greatly.

These data show clearly that in spectrophotometric studies of bilirubin in blood serum the possibility of errors caused by traces of oxyhemoglobin cannot be ignored, even in serums which appear free from red color. Corrections must be made for the effects of the oxyhemoglobin unless these effects can be shown to be negligible.

DETERMINATION OF CONCENTRATION OF TRACES OF OXYHEMOGLOBIN

The concentration of hemoglobin in traces of oxyhemoglobin in the serum was measured with a Keuffel and Esser spectrophotometer ("color analyzer") by the method described by the authors.⁶ This method, while accurate to 1 or 2 per cent for the concentrations of hemoglobin usually found in blood, is much less accurate for very low concentrations. But the accuracy is sufficient to permit of fairly reliable corrections for absorption by traces of oxyhemoglobin.

Another method, of about equal reliability for very low concentrations of hemoglobin, was used on some of the serums. The serum was diluted a known amount, shaken with air, and examined in a glass cell of adjustable thickness placed in front of the slit of a spectrometer. With light from an incandescent lamp (always the same lamp and voltage) passing through the cell, the thickness of serum was reduced until the absorption band of oxyhemoglobin at wave length 577 $m\mu$ disappeared. From the thickness at disappearance of the band, the concentration of hemoglobin was found by comparison with values obtained for known concentrations.

ABSORPTION SPECTRUM OF BILIRUBIN

To determine concentrations of bilirubin spectrophotometrically, it is necessary to measure the transmission only at one suitable wave length for which the absorption ratio is known. But in order to identify or otherwise investigate the pigment, one usually must determine the curve which represents the absorption spectrum. This may be represented by plotting absorptions, extinction coefficients or absorption ratios. Or transmissions may be plotted, since the values of transmission and of absorption are complementary. We shall plot transmissions.

So far as possible we shall reserve for a separate paper the discussion of the absorption spectra we have determined for various samples of bilirubin.

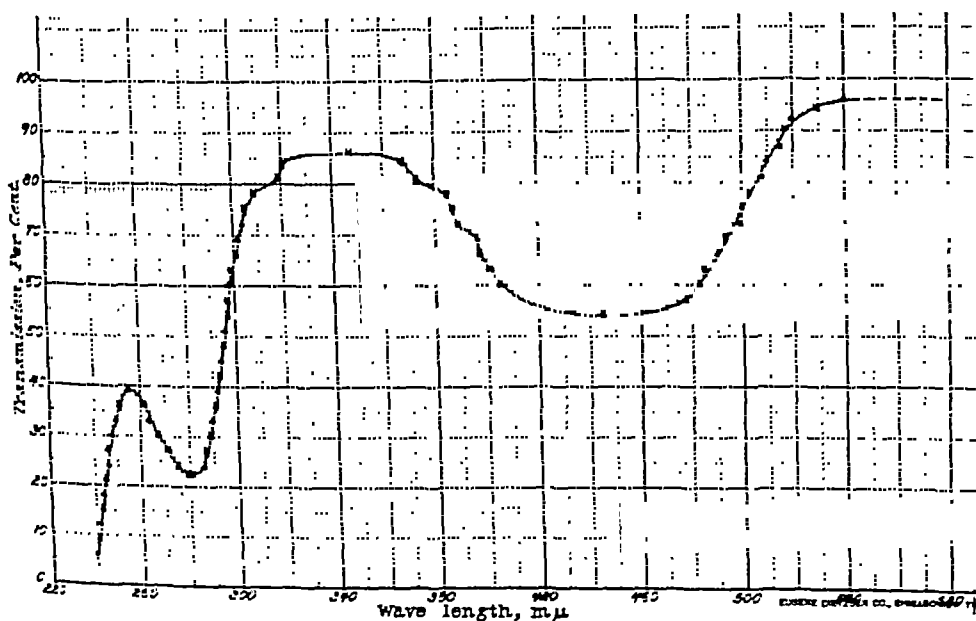


Fig. 1.—Spectrum of blood serum of jaundiced human being. For wave lengths longer than about 340 $m\mu$, the spectrum is typical of serum bilirubin. Concentration of bilirubin, 18.6 mg. per 100 c.c. Serum undiluted. Thickness, 0.128 mm.

However, to illustrate the general characteristics of the spectrum, we show in Figs. 1 and 2 the curves obtained for blood serums from two jaundiced patients. Distilled water was used as comparison substance, since bilirubin-free serum was not available, for reasons already stated. Hence the curves must show the absorption due not only to bilirubin but also to other substances in the serum. However, at wave lengths longer than 330 or 340 $m\mu$ no substance ordinarily present in serum, excepting hemoglobin or its compounds, is at all comparable to bilirubin in its power to absorb radiation. Therefore above 330 or 340 $m\mu$ the curves represent the absorption spectrum of bilirubin fairly closely, particularly in Fig. 1, where the concentration is very high. In Fig. 2 correction was made for the absorption due to oxyhemoglobin (upper curve), the broken line curve representing the corrected bilirubin spectrum. The curve in Fig. 1 needed no correction, since no oxyhemoglobin appeared in the serum.

The prominent band with maximal absorption at $280\text{ m}\mu$ is not characteristic of bilirubin. This is shown by the curves we have obtained for various samples of serum, from which it appears that the absorption in this region is independent of the concentration of bilirubin. Lewis¹¹ found the same band in the spectra of pseudoglobulin, euglobulin and albumin, from which he concluded that its appearance in the spectrum of blood serum is due specifically to the serum proteins. Later Stenström and Reinhard^{21, 22} decided from spectrophotometric evidence that the tryptophane and tyrosine constituents of the protein molecule are responsible for this band.

The curves in Figs. 1 and 2 are alike in general shape but differ in minor details. For the most part the minor differences are not due to experimental

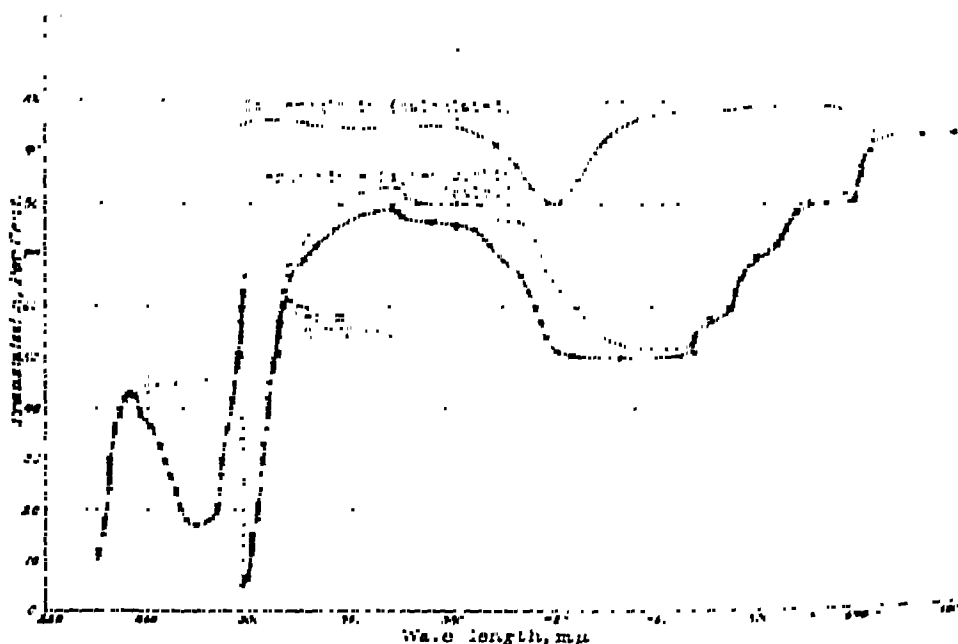


Fig. 2.—Spectrum of blood serum of jaundiced human being. For wave lengths longer than about $340\text{ m}\mu$, the general shape of the curve (broken line) is typical of serum bilirubin. The lower curve represents observed transmissions. The upper curve is calculated, to represent the trace of oxyhemoglobin present in the serum and is used in obtaining the corrected curve (broken line). Bilirubin concentration, $4.3\text{ mg. per } 100\text{ c.c.}$ Serum undiluted. Thickness, above $298\text{ m}\mu$, 0.772 mm.

errors. The prominent feature of both curves, definitely characteristic of bilirubin, is the broad absorption band extending from the green region of the visible spectrum to the neighborhood of wave length $360\text{ m}\mu$ in the ultraviolet. The wave length of maximal absorption seems to vary somewhat. It is difficult to locate accurately, since the absorption band is very broad and rather "flat" at its lower end. In Figs. 1 and 2 maximal absorption is approximately at wave lengths 445 and $460\text{ m}\mu$, respectively. Practically all our curves for bilirubin in human blood serum gave evidence of maximal absorption at some wave length between these two values, the average being about $453\text{ m}\mu$. Rahier¹⁵ located it at $435\text{ m}\mu$ but did not state whether his observations were made on serum bilirubin or on solutions of purified bilirubin. We have found an aver-

age value of 432 $m\mu$ for purified bilirubin dissolved in 0.02 N sodium hydroxide, this value being the average of nine determinations.

ABSORPTION SPECTRUM OF OXYHEMOGLOBIN

The absorption spectrum of oxyhemoglobin, Fig. 3, was obtained from samples of blood from three normal men. Clotting was prevented by adding a little dry ammonium oxalate. The hemoglobin content was measured by the spectrophotometric method of the authors⁶ and was found to be normal in each case. Part of each sample of blood was centrifuged and the plasma used as fluid for comparison. The whole blood and plasma were diluted equally with 0.1 per cent sodium carbonate solution. After dilution the concentration of hemoglobin was 1.22 mg. per c.c. This solution was shaken with air to convert the

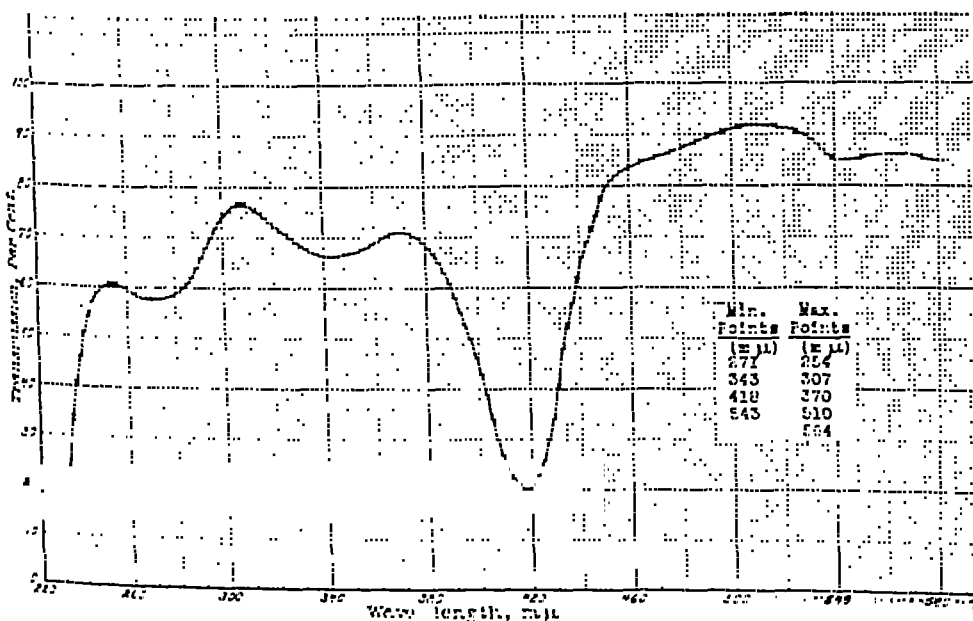


Fig. 3.—Spectrum of oxyhemoglobin normally present in human blood serum, computed for an assumed transmission of 30 per cent at wave length 427 $m\mu$.

hemoglobin into oxyhemoglobin. A layer 0.772 mm. thick was examined in each case.

Since the diluted blood was not centrifuged, the absorption represented in Fig. 3 includes that due to the stroma of the hemolyzed cells. The absorption by these cell bodies is small compared to that of the oxyhemoglobin, however. The following facts justify this statement: (1) Curves obtained by Strub²³ show this to be the case in the spectral range between wave lengths 450 and 660 $m\mu$. (2) In direct spectrophotometric comparisons of blood serums we have obtained several curves representing the spectrum of oxyhemoglobin alone. These agree closely with the curve in Fig. 3, from wave length 320 or 340 to 580 $m\mu$.

The curves from the three blood samples were exactly alike in general shape. Corresponding transmissions differed by less than the experimental

errors of measurement, over a major part of the spectral range represented. Positions of maxima and minima, 9 in all, agreed on the average to within 0.7 $m\mu$.

To calculate the curve of Fig. 3, a wave length in the principal absorption band was first chosen, at which wave length, 427 $m\mu$, all three curves indicated the same transmission. The optical densities ($-\log_{10} T$) at a considerable number of other wave lengths were each divided by the density at 427 $m\mu$ and at each wave length the average value of the three density ratios was used in calculating the transmission, arbitrarily assuming a value of 30 per cent at 427 $m\mu$. This procedure is based on the fact, previously stated, that for any two

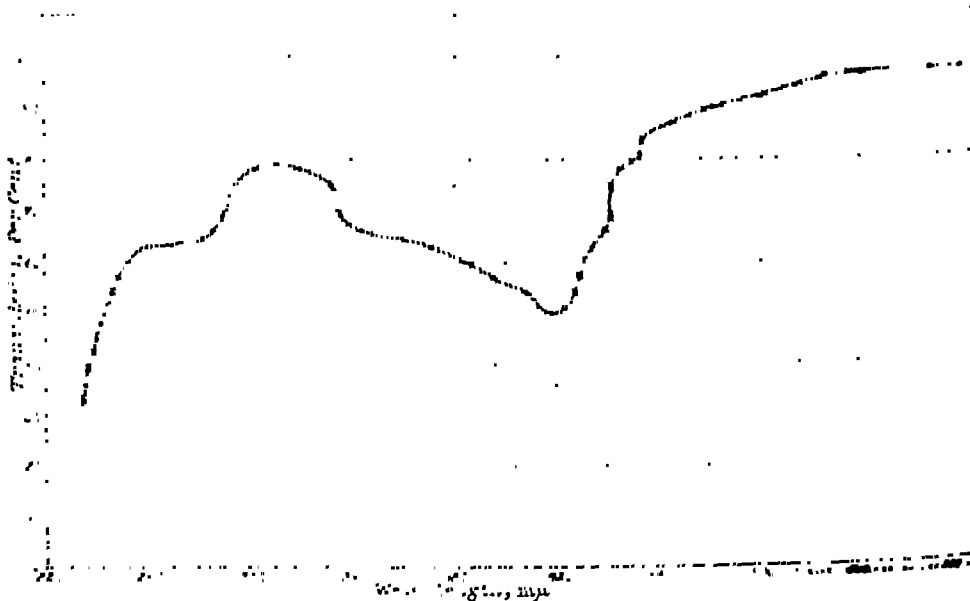


Fig. 4.—Spectrum of oxygenated solution of purified hemoglobin dissolved in water containing a little ammonium hydroxide. Concentration of hemoglobin, 0.00121 gm. per c.c. Thickness, 0.772 mm. The α - and β -bands, at 577 and 542 $m\mu$, respectively, were visually observed but are not shown here because of their faintness and the uncertainty in measuring transmissions near 100 per cent in this spectral region.

wave lengths the ratio of optical densities is a constant independent of concentration and thickness. Some of the average density ratios are shown in Table II.

For comparison with Fig. 3 we show in Fig. 4 the transmission curve for a solution of purified hemoglobin in water containing a little ammonium hydroxide (1 part of fairly concentrated ammonium hydroxide to 125 parts of water). The hemoglobin was prepared by the method of Welker and Williamson. In the solution the concentration of hemoglobin was 1.21 mg. per c.c. The solution was examined spectrophotometrically within thirty minutes after preparation and was not exposed to radiation which might cause it to "fade." The solution was shaken with air just before examination. We are not prepared to say whether the resulting compound of hemoglobin was oxyhemoglobin or a salt of hemoglobin. However, we note that when sodium hydroxide was substituted for the ammonium hydroxide, visual spectroscopic examination revealed a broad

TABLE II

SOME OF THE DENSITY RATIOS OF OXYHEMOGLOBIN USED IN COMPUTING THE CURVE OF FIG. 3

WAVE LENGTH λ (m μ)	DENSITY RATIO ($= -\log_{10} T_{\lambda} / -\log_{10} T_{427}$) [*]
235	0.896
245	0.460
254	0.416
270	0.459
290	0.360
307	0.221
320	0.267
340	0.339
360	0.310
370	0.284
380	0.315
390	0.430
400	0.664
405	0.893
410	1.15
418	1.33
427	1.000
433	0.606
442	0.328
450	0.194
460	0.151
480	0.114
510	0.071
525	0.081
542	0.136
565	0.122
580	0.135

^{*}Numerator and denominator represent the optical densities at wave lengths λ and 427 m μ , respectively.

absorption band centered at about wave length 620 m μ , in addition to the usual α - and β -bands at 577 and 542 m μ , shown by both solutions. The α - and β -bands were less distinct than in the solution with ammonium hydroxide. (In Fig. 4 these bands are not indicated, for reasons noted under the figure.) These differences show that the nature of the compound of hemoglobin obtained in such a solution depends on the kind of alkali which is added.

It is seen by comparing Figs. 3 and 4 that although the two spectra are generally similar, the spectrum in Fig. 4 differs from that in Fig. 3 in the following particulars: (1) considerably greater absorption in the spectral region lying approximately between wave lengths 340 and 395 m μ ; (2) very much less absorption in the principal part of the most prominent absorption band, roughly from 395 to 440 m μ ; (3) less absorption in the region of the α - and β -bands, 577 and 542 m μ . These differences could not be due to the stroma in the serum, since these bodies would increase rather than decrease the absorption shown in Fig. 3, which action could not cause the first difference, and since the stroma does not absorb specifically in the spectral regions of the three bands mentioned in the statement of the second and third differences. A reliable quantitative comparison of the two curves may be made by comparing corresponding ratios of optical density. Some such ratios with respect to the density at wave length 460 m μ are shown in Table III. Wave lengths of maximal and minimal absorption up to 510 m μ are represented, with some inter-

mediate values. While at each wave length the magnitude and algebraic sign of the difference shown in the last column are dependent on the choice of wave length at which the optical density is taken as reference, the great range in magnitudes and the wide departures from zero show without ambiguity that the two spectra cannot belong to identical substances, although the substances evidently are rather closely related chemically. This would be true also if sodium hydroxide were substituted for the ammonium hydroxide in preparing

TABLE III
COMPARISON OF DENSITY RATIOS FOR SPECTRA OF FIGS. 3 AND 4

WAVE LENGTH λ ($m\mu$)	DENSITY RATIO ($= -\log_{10} T_{\lambda} / -\log_{10} T_{460}$) *		DIFFERENCE, IN PER CENT OF VALUE FOR FIG. 3
	FIG. 3	FIG. 4	
235	5.98	7.32	+22
254	2.76	3.35	+21
271	3.03	3.08	+2
307	1.45	1.60	+10
343	2.25	2.78	+24
370	1.88	3.10	+65
395	3.60	3.80	+6
418	8.77	4.69	-47
430	5.51	3.50	-36
442	2.17	1.86	-14
460	1.000	1.000	0
510	0.465	0.432	-7

* Numerator and denominator represent the optical densities at wave lengths λ and 460 $m\mu$, respectively.

the compound of hemoglobin, as is shown by the nature of the resulting modifications observed in the absorption spectrum. Therefore we conclude that the oxyhemoglobin normally present in blood is not identical with the compound formed by oxygenating a solution of purified hemoglobin in water containing a small amount of ammonium hydroxide or of sodium hydroxide.

The curves in both Figs. 3 and 4 gave evidence of a very strong absorption band, less strong in Fig. 4, with maximal absorption at 418 $m\mu$. Two other conspicuous bands appear in the ultraviolet, with maximal absorption located at or very near wave lengths 271 and 343 $m\mu$ in Fig. 3 but much less definitely indicated as to position in Fig. 4. In Fig. 4 the separation of the bands at 418 and at or near 343 $m\mu$ is masked by the absorption in the region between them. Both curves show a cut-off at about 230 $m\mu$.

Few accurate data have been published on the absorption spectrum of oxyhemoglobin in the ultraviolet and shorter wave lengths of the visible spectrum. Kayser¹⁰ refers to a number of observations made by various investigators between 1878 and 1904, but many of the data secured are unreliable or are expressed only in approximate figures. Strub²³ has published absorption curves and data for the visible region above 450 $m\mu$. Suhrmann and Kollath²⁴ show absorption curves for solutions of blood, in general shape closely similar to the curve in Fig. 3 and with the positions of the absorption bands approximately the same. But only eight values are plotted in the entire spectral range between 315 and 580 $m\mu$, so that the shape of the curve and the positions of maxima

and minima as shown can be considered only as rough approximations. Hicks and Holden⁶ have published values of the wave lengths at which maximal and minimal absorption occur in solutions of oxyhemoglobin in the ultraviolet and violet spectrum. The oxyhemoglobin was prepared by the method of Adair. In the principal band, maximal absorption was observed at 411.5 $m\mu$. Berg and Schwarzaeher¹ located maximal absorption at 413.7 $m\mu$. Details of their experimental work are not given.

As shown in Fig. 3, we find that in the case of oxyhemoglobin occurring normally in human blood serum, maximal absorption in the principal absorption band occurs at 418 $m\mu$. This location is clearly indicated in a considerable number of other spectra which we have charted. Early observers, for the most part employing less accurate methods of measurement, usually assigned some value between 408 and 415 $m\mu$. In treatises on physiologic chemistry and in other recent writings, one of these earlier values usually is quoted, apparently without verification. In the absorption curves obtained by Suhrmann and Kollath, maximal absorption is indicated at about 408 $m\mu$. But the curves are only rough approximations, for reasons already stated. The few points which are plotted fall more logically on a curve showing maximal absorption at 415 or 420 $m\mu$. In the measurements by Hicks and Holden, who found maximal absorption at 411.5 $m\mu$, it is to be remembered that their results are for solutions of purified oxyhemoglobin. In view of our demonstration of the lack of identity between oxyhemoglobin normally present in blood and the compound of hemoglobin formed by oxygenating a solution of purified hemoglobin in water containing a small amount of ammonium hydroxide or sodium hydroxide, it cannot be assumed a priori that the oxyhemoglobin examined by Hicks and Holden has an absorption spectrum identical with that of oxyhemoglobin occurring normally in blood.

The average value of the absorption ratio of oxyhemoglobin at wave length 418 $m\mu$, as computed from the three curves from which Fig. 3 was calculated, is 0.000,136 gm. per sq. cm., where the concentration is in gm. per c.c. and the thickness in cm. At 427 $m\mu$ the value is 0.000,181. Using these values and the density ratios in Table II to calculate the absorption ratio at 542 $m\mu$, the wave length of maximal absorption in the β -band, the value comes out 0.00133, not in close agreement with the value 0.00110 previously found by the authors.⁶ But 0.00133 is much less reliable, being based on curves showing transmissions of 82, 85, and 88 per cent, respectively, at wave length 542. At high transmissions (T) the optical densities ($-\log_{10} T$) are small and vary rapidly with changes in T. Therefore the accuracy of the determined values of optical density and corresponding density ratios is affected to a disproportionately large degree by errors in T, the effect increasing as T approaches 100 per cent. The curve showing T = 82 per cent at 542 $m\mu$ gives an absorption ratio of 0.00107, in good agreement with the value found previously.

METHOD OF CORRECTING FOR ABSORPTION BY OXYHEMOGLOBIN IN DETERMINING THE ABSORPTION SPECTRUM OR CONCENTRATION OF BILIRUBIN

We have presented data which clearly show the necessity of making corrections for the absorption due to traces of oxyhemoglobin when investigating serum

bilirubin spectrophotometrically. If only the concentration of the bilirubin is to be determined, it is only necessary to find the transmission of the oxyhemoglobin at one wave length, namely, that wave length at which the transmission of the bilirubin is used in calculating its concentration. But if the nature of the investigation makes it necessary to map the absorption spectrum of the bilirubin, the transmission of the oxyhemoglobin must be determined at a sufficient number of wave lengths to make it possible to correct the observed transmission spectrum throughout its length. In either case the transmission of the oxyhemoglobin at any wave length shorter than about 520 or 530 $m\mu$ must be calculated from its concentration, since the bilirubin interferes with direct measurement of the oxyhemoglobin transmission. The two methods of estimating traces of oxyhemoglobin have been presented and discussed. The procedure to be followed in making the corrections will be made clear by the following illustration:

In Fig. 2, the portion of the curve labeled "serum" lying between wave lengths 340 and 580 $m\mu$ is the typical uncorrected curve for serum which contains an abnormally high concentration of bilirubin. The hemoglobin concentration (C) was estimated to be 0.000,17 gm. per c.c., an amount too small to be detected by the unaided eye. The thickness (L) of serum was 0.0772 cm. The absorption ratio (A) of oxyhemoglobin at 427 $m\mu$ (the choice of wave length is arbitrary, providing we use the proper absorption ratio) is 0.000,181 gm. per sq. cm. Therefore at this wave length the transmission T_{427} is found by substitution in Equation (1), which gives

$$0.000,17 = - \frac{0.000,181}{0.0772} \log_{10} T_{427},$$

from which $T_{427} = 0.846$ or 84.6 per cent. The transmissions at other wave lengths are found from this value by the use of Table II. For example, at 380 $m\mu$ we have, from the table,

$$\frac{-\log T_{380}}{-\log T_{427}} = 0.315.$$

Since $T_{427} = 0.846$, this gives $T_{380} = 0.949$ or 94.9 per cent. Plotting this value and others similarly found, the oxyhemoglobin curve shown in the figure is drawn. To correct the observed spectrum (curve marked "serum"), the transmission at each of a number of different wave lengths is divided by the corresponding transmission shown by the oxyhemoglobin curve. For example, at 430 $m\mu$ the respective transmissions are 50.3 and 87.1 per cent, therefore the corrected value is 50.3/87.1 or 57.7 per cent. This value and others similarly determined are plotted to obtain the dotted curve labeled "serum minus oxyhemoglobin," which represents the corrected bilirubin spectrum between wave lengths 340 and 580 $m\mu$.

In case the oxyhemoglobin transmission is first calculated for some wave length, λ_1 , other than 427 $m\mu$, the density ratios in Table II cannot be used. Instead, new values are found by dividing each tabulated value by the value given for λ_1 . For example, if λ_1 is 418 $m\mu$, the density ratio with respect to it will be, at 400 $m\mu$, 0.664/1.33 or 0.499; at 450 $m\mu$ it will be 0.194/1.33 or 0.146; and so on.

If it is desired only to find the concentration of the bilirubin, it is unnecessary to determine the entire oxyhemoglobin curve. Only one value of transmission is needed. For example, if the transmission of the serum is determined at wave length 410, it is necessary to know the transmission of the oxyhemoglobin only at that wave length. The method of finding it is illustrated above. The correction is made by dividing the observed transmission of the serum at 410 $m\mu$ by the corresponding transmission of the oxyhemoglobin. The corrected value is used in calculating the bilirubin concentration by means of Equation (1).

Such corrections may seem somewhat laborious. But if serum bilirubin is to be studied spectrophotometrically, either the corrections must be made or it must be shown that oxyhemoglobin is not present in amount sufficient to alter the spectrum significantly. So far as oxyhemoglobin in serum is concerned, the unaided eye is incapable of deciding whether the serum is spectrally "clear" or not.

DETERMINATION OF CONCENTRATION OF BILIRUBIN

The concentration of bilirubin is found by substituting in Equation (1) the values of A, L, and T. It is best to determine the transmission T at some wave length in the most prominent absorption band, preferably at or near the wave length at which maximal absorption occurs. In the case of bilirubin in human blood serum, we have found this wave length to be approximately 453 $m\mu$. The exact value is immaterial, since the absorption band is broad and the transmission near the minimum varies but little over a considerable spectral range (Figs. 1 and 2). Minimal transmission in the narrower oxyhemoglobin band is at 418 $m\mu$. Therefore the error due to absorption by traces of oxyhemoglobin may be largely avoided by measuring the transmission at 450 or 460 $m\mu$, since at these wave lengths the absorption by oxyhemoglobin is very much less than at 418 $m\mu$ (Fig. 3).

In Table IV are shown the values of the absorption ratios of bilirubin at wave lengths 450 and 460 $m\mu$, calculated from transmission curves obtained in our investigations of the two types of bilirubin in human blood serum and in our observations on the development of obstructive jaundice in the dog. All the serums were undiluted when examined, except the second, fifth, and sixth, which were diluted to one-third the concentration shown in the table by adding 0.1 per cent sodium carbonate solution.

The serums were prepared as follows: The blood was carefully drawn and placed in an ice box until clotted, after which the clot was loosened and the serum obtained by centrifuging. The serum was returned at once to the ice box or put in a Dewar flask containing ice at 0° C., where it was left until its spectrophotometric examination, which was made within a few hours. Since the serum was kept in the dark and at 0° or a little above, "fading" probably was inappreciable.

In averaging the values of the absorption ratios (A, Table IV), each was assigned a weight proportional to the reciprocal of the error in A resulting from an error of 1 per cent transmission in determining the transmission. Such

error in A becomes progressively larger as the transmission approaches 0 per cent or 100 per cent and is smallest in the range from about 20 per cent to 60 per cent.

The individual values of A in Table IV admittedly vary too much to warrant regarding the averages as highly accurate. To a small extent the variations are due to inaccuracy of the spectrophotometric determinations. But careful consideration has led us to conclude that the variations are due mostly to errors in the values of bilirubin concentration C as determined by the van den Bergh method. In spite of the large average deviations, the average values of A obtained from blood serum of man and of dog are in close agreement. The differences (3.5 and 1.8 per cent) are too small to be considered as other than experimental. The values for the two kinds of serum probably are identical. Also, for each serum the average values at wave lengths 450 and 460 $m\mu$ are very nearly equal. For either wave length and for the serum either of man or of dog we may consider 11.3×10^{-6} or 0.000,011,3 gm. per sq. cm. as the correct value of the absorption ratio of bilirubin. This agrees closely with the value 11.4×10^{-6} found by Cutten, Emerson and Woodruff² for bilirubin dissolved in chloroform, at wave length 450 $m\mu$.

It is recommended that in determining the concentration of bilirubin in blood serum the transmission be observed at wave length 450 or 460 $m\mu$. The latter is preferable, since at this wave length oxyhemoglobin absorbs a little less radiation than at 450 $m\mu$. The absorption ratio for oxyhemoglobin at 460 $m\mu$, calculated from Table II and the value 0.000,136 at 418 $m\mu$, is 0.001,20 gm. per sq. cm.

TABLE IV

VALUES OF THE ABSORPTION RATIOS (A_{450} AND A_{460}) OF BILIRUBIN IN BLOOD SERUM, AT WAVE LENGTHS 450 AND 460 $m\mu$. C IS THE CONCENTRATION OF BILIRUBIN AS DETERMINED BY THE VAN DEN BERGH METHOD

HUMAN SERUM			DOG SERUM		
C (mg. per 100 c.c.)	A_{450} (gm./cm. ²)	A_{460} (gm./cm. ²)	C (mg. per 100 c.c.)	A_{450} (gm./cm. ²)	A_{460} (gm./cm. ²)
1.7	8.5×10^{-6}	8.6×10^{-6}	1.6	5.0×10^{-6}	4.8×10^{-6}
9.8	13.7	14.0	2.0	5.1	5.1
4.3	11.6	11.5	4.5	14.5	14.4
4.5	11.5	11.4	5.0	10.4	10.5
15.0	9.1	9.1	6.0	9.8	9.7
15.0	8.5	8.5	8.1	17.1	16.8
22.3	11.8	12.4	9.2	17.5	17.3
23.7	16.8	17.1			
Weighted average	11.1×10^{-6}	11.2×10^{-6}		11.5×10^{-6}	11.4×10^{-6}
Average deviation	2.1×10^{-6}	2.2×10^{-6}		4.3×10^{-6}	4.2×10^{-6}
Weighted average of all values of $A_{450} = 11.3 \times 10^{-6}$ gm. per cm. ² .					
Weighted average of all values of $A_{460} = 11.3 \times 10^{-6}$ gm. per cm. ²					

SUMMARY AND CONCLUSIONS

The ultraviolet and visible absorption spectrum of bilirubin in blood serum was investigated by means of a quartz spectrograph and rotating sector photometer, with an under-water spark as source of radiation. Data were obtained

on the visibility of small amounts of oxyhemoglobin in water and in blood serum, and on the effects of traces of this pigment in spectrophotometric investigations of the components of serum, particularly bilirubin. Characteristic spectra of serum bilirubin, of serum oxyhemoglobin and of an oxygenated solution of purified hemoglobin in alkalized water are shown and discussed. Some constants of the absorption spectra of serum oxyhemoglobin and of serum bilirubin are evaluated. A method of calculating the spectrum of a small amount of oxyhemoglobin in serum and of correcting the observed spectrum is described. The principal conclusions are as follows:

1. In blood serum, traces of oxyhemoglobin not detectable by the unaided eye may absorb considerable amounts of radiation, modifying the absorption spectrum greatly.
2. In spectrophotometric investigations of bilirubin in blood serum, corrections must be made for absorption of radiation by traces of oxyhemoglobin, even in serums which appear "clear," unless it can be shown that such absorption is negligible.
3. In correcting the absorption spectrum of blood serum or of other body fluid for the absorption due to oxyhemoglobin, it is necessary to determine the concentration of oxyhemoglobin and then calculate its transmission (a) at various wave lengths, in case the nature of the spectrum is in question; or (b) at one particular wave length only, in case only the bilirubin concentration is required.
4. The most prominent absorption band of serum bilirubin is very broad. In human serum, minimal transmission in this band occurs at or near $453\text{ m}\mu$. Minimal transmission in the most prominent absorption band of oxyhemoglobin in serum is at $418\text{ m}\mu$.
5. The oxyhemoglobin normally present in blood is not identical with the compound formed by oxygenating a solution of purified hemoglobin in water containing a small amount of ammonium hydroxide or of sodium hydroxide.
6. Serum oxyhemoglobin has the following absorption ratios, in gm. per sq. cm.: at $418\text{ m}\mu$, 13.6×10^{-6} ; at $427\text{ m}\mu$, 18.1×10^{-6} ; at $460\text{ m}\mu$, 12.0×10^{-6} . The absorption ratios of serum bilirubin at 450 and $460\text{ m}\mu$ are very nearly equal, both having approximately the value 11.3×10^{-6} gm. per sq. cm., both in the serum of man and of dog.
7. It is recommended that in spectrophotometric determinations of the concentration of bilirubin in blood serum, the transmission be observed at wave length $460\text{ m}\mu$.

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A COMPARISON OF BLOOD SUGAR CURVES WHEN GLUCOSE IS GIVEN ORALLY AND INTRADUODENALLY*

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THE glucose tolerance test is a common clinical diagnostic aid, but its value depends upon a knowledge of the various processes concerned in the response and upon a careful control of the factors which influence its interpretation. These factors include the previous period of fasting, the source of the blood used and the amount of exercise taken during the test. Since the absorption of glucose by the stomach is negligible¹ it seems possible that the emptying rate of the stomach might also influence the curve obtained.

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In support of this hypothesis John² observed that nausea produced "peculiar dips" in certain blood sugar curves which he interpreted to mean that absorption was delayed or stopped for a short time. Beeler, Bryan, Cathart and Fitz,³ however, noted that after the ingestion of 100 gm. of glucose by subjects without evident gastric disease, from 26 to 68 per cent of the glucose was still in the stomach at the end of an hour. In the one case in which blood

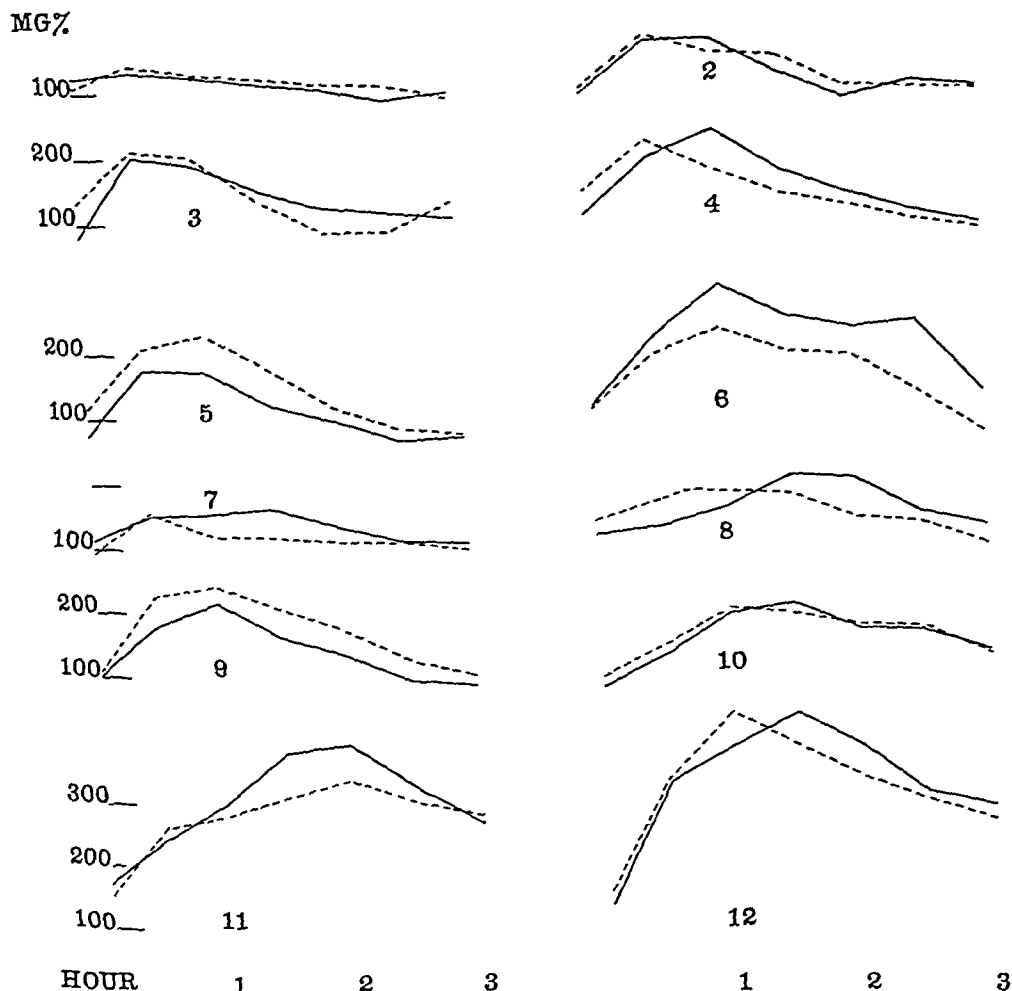


Chart 1.—Blood sugar curves when glucose is given orally and intraduodenally.
—Oral curve. ---- Duodenal curves.

sugar figures were reported, the curve reached a peak in thirty-five minutes and had nearly returned to the fasting level in one hour. The stomach at this time contained 27 gm. of glucose, an amount which if given to a fasting person would increase the blood sugar. This may be explained by the work of Cori⁴ who has shown that glucose is absorbed from the intestine at a constant rate which within wide limitations is independent of the concentration; and that even after a moderate carbohydrate meal glucose absorption is at a

maximum. He states:⁵ "No indication was obtained, as in the case of alcohol, that emptying of the stomach is a limiting factor (in absorption) though perhaps more work is required to settle this problem definitely."

To what extent then does the emptying of the stomach influence the interpretation of the blood sugar curve? Earlier work on this question was done by Hale-White and Payne⁶ who reported an experiment on one subject to whom they gave glucose into the duodenum at various rates. When 50 gm. was given within eleven minutes, the blood sugar curve was almost identical with the one obtained when glucose was given by mouth. When the rate of administering the glucose was decreased so that the time required was one and five-tenths hours, it was found that the rise was almost identical with the other two curves, that the duration of hyperglycemia was greatly increased and that the descent was similar in all instances. They concluded from this case that the emptying rate of the stomach probably does not influence the initial rise of the blood sugar, but that it may change the period of hyperglycemia.

It was believed that the clinical significance of the emptying rate of the stomach could be more adequately ascertained by comparing blood sugar curves obtained when glucose was given directly into the duodenum with those obtained when glucose was given by mouth. For this study 12 subjects were chosen at random from the hospital wards. Two tests were made on each subject at an interval of not less than three days. The test when glucose was given by mouth was conducted as usual. After a fourteen-hour fast the subjects were given 50 gm. of glucose in a 15 per cent solution as that was the concentration found³ to pass most quickly from the stomach. The subjects drank the solution as rapidly as possible, the ingestion requiring less than a minute. Finger blood for sugar determination was obtained before the ingestion of the glucose and for each half hour thereafter for three hours. Blood sugar was determined by means of the Boyd-Gibson micromodification of the Folin-Wu method.⁷

For the duodenal tests a Rehfuß tube was given. It was considered that the bucket was in the duodenum when a bile-stained alkaline fluid was obtained by aspirating. The fasting blood sugar was not taken until after the tube was in the duodenum, so that if any change occurred in the blood sugar due to the swallowing of the tube it could be considered the fasting level. Warmed glucose solution of the same concentration as previously given by mouth was then given, the administration usually taking from three to five minutes. Blood samples were taken as before.

The data obtained are given in Table I and Chart 1. It is evident that the fasting level of the duodenal curves is slightly higher than the oral curves in 7 of the 12 cases. The height of the peak in the two types of curves shows no consistent relationship, the duodenal peaks are higher in 5 cases, lower in 4 cases and the same as the gastric curves in 3 cases. At the end of the three hours the blood sugar values of each pair of curves approximate each other very closely.

TABLE I

A COMPARISON OF BLOOD SUGAR CURVES WHEN GLUCOSE IS GIVEN ORALLY AND INTRADUODENALLY

CASE	SEX	AGE	WT. KG.	DIAGNOSIS	BLOOD SUGAR MG. PER CENT						
					0	1½	1	1½	2	2½	3
1	M	16	41	Colitis	123	133	127	115	104	86	96
					*110	142	130	120	111	105	86
2	M	20	65	Fragile bones	87	163	166	113	76	96	86
					95	173	147	138	91	84	80
3	M	16	54	Dental caries	81	212	194	151	123		104
					135	213	201	135	85	85	129
4	M	34	67	Arthritis	102	186	228	165	129	100	79
					142	213	165	129	110	83	70
5	M	41	53	Neurasthenia	79	179	175	122	97	64	70
					119	213	232	179	118	83	73
6	F	37	45	?	112	214	290	243	224	234	128
					110	190	226	190	182	128	66
7	M	37	76	Splenic anemia	114	153	155	162	135	110	105
					93	155	122	120	110	110	97
8	F	64	45	Diabetes insipidus	113	126	153	200	198	147	122
					138		181	175	137	129	94
9	M	53	88	Mild diabetes	105	182	218	164	135	92	84
					108	228	243	208	172	126	100
10	M	56	54	Mild diabetes	80	127	190	203	168	165	132
					93	140	198	190	170	165	127
11	M	22	73	Diabetes	172	243	300	380	391	324	268
					151	260	280	311	336	300	280
12	M	36	64	Diabetes	138	324	385	434	385	311	291
					158	336	434	385	336	300	270

*The second series is the duodenal curve in each case.

It is apparent from the charted curves that the general shape of the two curves for each patient is quite similar. One curve may run consistently higher or lower than the other; when there is a tendency toward a plateau, it is evident in both curves.

SUMMARY

Blood sugar curves following the administration of 50 gm. of glucose by mouth and intraduodenally show no consistent difference.

It is concluded, therefore, that the emptying rate of the stomach ordinarily has little or no effect on the glucose tolerance test.

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ELLIPTICAL RED BLOOD CELLS IN MAN*

A REPORT OF ELEVEN CASES

SARAH H. McCARTY, A.B., BIRMINGHAM, ALA.

IN HEALTH the erythrocytes of man exhibit such a remarkably constant shape that it is of considerable interest when the microscopist encounters an individual whose erythrocytes in a large proportion of their numbers appear not as the usual biconcave discs we are accustomed to see, but as flattened ellipsoids. This paper is a report of eleven such cases. Four of them were discovered in the routine microscopic examination of bloods from approximately 10,000 individuals. The other seven cases are relatives of three of these four patients.

HISTORICAL

During the past twenty-nine years this anomaly has been observed in more than sixty individuals. The first case was reported by Dresbach^{1, 2} in 1904. The subject was a young medical student who was apparently in good health at the time. The observation occasioned considerable scientific debate, since the young man died shortly thereafter of a rheumatic heart. One of Dresbach's contemporaries³ insisted that the poikilocytosis was the result of an obscure form of pernicious anemia or purpura, but Ewing, Erlich and other noted pathologists came to the conclusion that it was an anomaly having no relation to a primary blood dyscrasia, since the red cell count and hemoglobin values were "up to standard." Ewald in a personal communication to Dresbach stated that a similar observation had been made at Königsberg twenty or thirty years previously.

The second case was reported by Bishop⁴ in 1914. The patient was a white male, aged forty-one, who exhibited no evidences of an anemia. Bishop was able to examine blood from members of his patient's family and found the erythrocytes of a sister similarly deformed.

Three cases were reported in 1923, one by Sydenstricker⁵ and two, a mother and daughter, by Huck and Bigelow.⁶

In 1927 Lawrence⁷ reported a series of eight cases of elliptical and sickle-shaped red cells. Since none of his patients exhibited a high percentage of deformed cells (5 to 10 per cent is the most reported and this patient suffered from a marked anemia) and since he makes no distinction between sickle cells and elliptical cells, though he pictures both, these cases have not been included in the total figures of this series.

In 1928 eleven cases of elliptical erythrocytes were reported from Germany and Holland, 5 by van den Bergh,^{8, 10} 1 by Bernhardt,⁹ and 5 by

*From the Hillman Hospital Laboratory.
Received for publication, April 20, 1933.

Günther.¹¹ Van den Bergh examined the family of his first patient, and found the father and two sisters similarly affected. These 3 individuals were healthy and had no anemia.

In 1929 Hunter and Adams¹² definitely established the familial nature of the phenomenon by observing the peculiarity in 3 generations of a family of Dutch extraction who had emigrated to the Northwest.

In 1931 Lawrence¹³ described 5 cases; 2 of the patients were the child and grandchild of one of his hospitalized patients. Later in the same year van den Bergh and Rehorst¹⁴ investigated the antecedents of the family K1..., the American branch of which had been described by Hunter and Adams. They found 7 other individuals similarly affected. Many of the relatives refused to have their blood examined or it is probable that the K1...s would have swollen the statistics on elliptical cells still further. In July, 1931, Roth and Jung¹⁵ described another case in a young white woman whose blood had been observed over a period of nine years with no appreciable change in the percentage of cells exhibiting the deformity.

In February, 1932, Terry and his associates^{16, 17} described two cases with autopsy. In March, 1932, Cheney¹⁷ reported the largest series of cases, 14 in all, in 3 generations of a family of Italian extraction.

In the last twenty-nine years, then (excluding the observations of J. S. Lawrence in 1927), the anomaly has been observed in 57 individuals and in 17 family groups. In addition there are known to be 11 cases not yet reported in the literature. Sydenstricker has observed 6 cases in addition to the one he reported, Jaffe has observed 2, and van den Bergh reports 2, one seen by Dr. Pelger of Amsterdam and one by Dr. Vroorn-Hilder. I include also the case at Königsberg with which Ewald was familiar. Males and females have been about equally affected. It has been seen in the mulatto, the American negro and in whites of Dutch, German, Italian, Scotch, and Hebrew extraction. The youngest individual known to have the peculiarity was aged nine months (see below) and the oldest was aged eighty-four (Günther's case¹¹).

CASE REPORTS

On only 3 of our cases was it possible to do detailed work. These patients remained in the hospital for some time and have been observed from time to time ever since. In the case of relatives showing the elliptical anomaly, it was practicable to make only microscopic blood examinations.

CASE 1.—B. T., a negress, aged twenty-five years, was admitted to the Hillman Hospital with typhoid fever. The Widal reaction was strongly positive, the leucocytes numbered 7,150. In making the differential blood count it was noted that 90 per cent of the red blood cells were peculiarly deformed, being about twice as long as they were broad. At this time we were investigating the incidence of meniscoeytosis among our hospital patients, and we prepared wet preparations of this blood in the usual manner. The striking elliptical deformity was present in these wet preparations also, but no "sickling" occurred. Repeated examinations of smears and cells collected in various fluids, Hayem's solution, Locke's solution, physiologic saline, citrate, and oxalate, always revealed the same abnormality. Blood has been collected from this woman from time to time during the last eight years. The hemoglobin values have ranged from 74 to 68 per cent (Sahli), the erythrocyte count from 4,630,000 to

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in the long axis of the cell. These cells resembled more closely than any others in this series the cells seen in the blood smear of a camel.

Hemoglobin values have ranged from 56 to 84 per cent (Sahli) in samples of blood taken at various times during the last five years. The erythrocyte counts have varied from

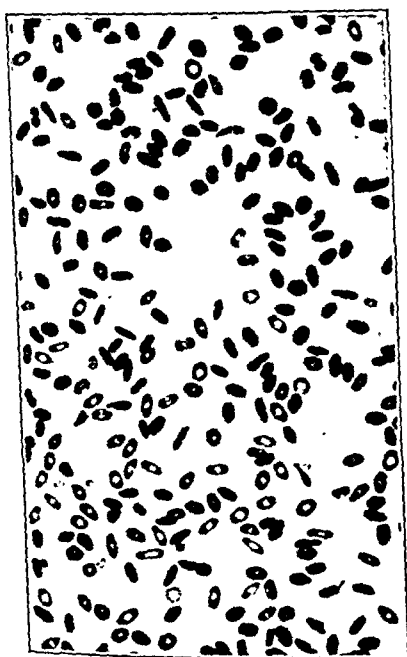


Fig. 2.—Stained film of blood of F. M.

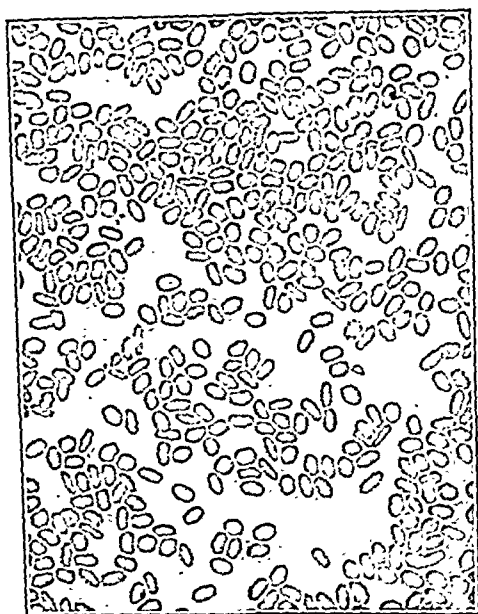


Fig. 3.—Wet preparation of blood of F. M.

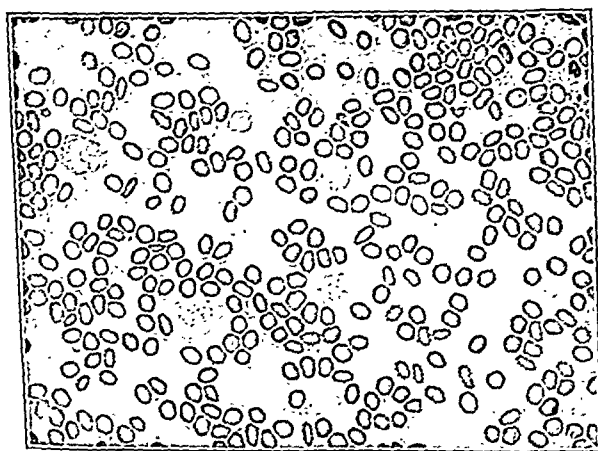


Fig. 4.—Wet preparation of blood of E. M., son of F. M. The percentage of elliptical cells is much less than in the mother's blood.

2,870,000 to 4,030,000 per c.mm. with a color index always of 1.1. The volume index was 1.04. It will be seen that although these cells are unusually long, their measurements for breadth and thickness are so diminished that they have very nearly a normal volume. "Fragility" test: initial hemolysis at 0.40 per cent NaCl, complete hemolysis in 0.32 per cent NaCl. Reticulocytes 0.3 per cent. Icteric index 9.

3,200,000 per c.mm. All hemoglobin values have been checked with the Van Slyke apparatus and counts have been made with U. S. Bureau of Standards pipettes. Except for a brief period when the patient was recovering from an anemia due to a severe menorrhagia, the color index has always been slightly greater than 1. In spite of this the cells in the smear appeared achromic. In examining wet preparations these pale-staining central areas appeared to represent biconcavities. In addition to the definitely elliptical cells there were many poikilocytes, sausage-shaped, bifid and tailed forms. The elliptical cells varied considerably in their dimensions. Micrometer measurements made on 1,000 cells in wet preparations gave average diameters of 10 by 4.5 by 2.7 micra. The thickness of the cell was hard to calculate, owing to the difficulty with which rouleaux were formed. Instead of stacking in rouleaux, the cells tended to group themselves in chains with the ends of the cells overlapping, similar to the phenomenon observed in camels' cells by Ponder.²⁵ Various methods for more accurate measurements were attempted, notably the Price-Jones method,^{23, 26} but this proved too elaborate for routine use, and it was found difficult to measure the fresh cells accurately even with the micrometer, owing to the movement of the cells in the suspending fluid. Following Haden's suggestion¹⁰ and working somewhat along the lines adopted by Emmons,²⁰ it occurred to us that the volume index might offer a good check on the size of the cells, since this would give us measurements of fresh cells in three dimensions. The volume index of this woman's blood was 1.23, as compared with a normal of 0.96.

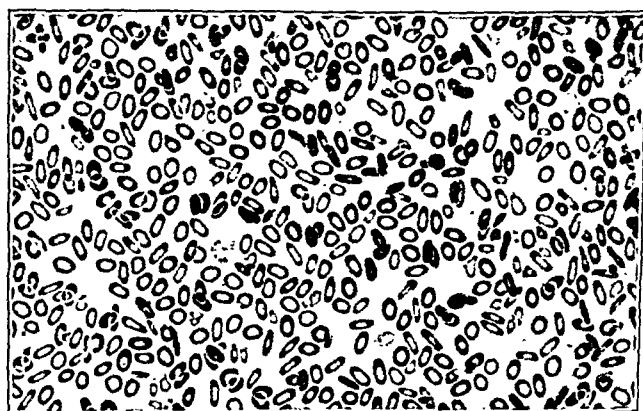


Fig. 1.—Stained film of blood of B. T. Tailed and bifid poikilocytes are seen.

The percentage of deformed cells was 91.1 in smears and 91.0 in wet preparations. These figures are based on counts of the number of poikilocytes occurring in 1,000 cells suspended in Hayem's fluid, and 1,000 fixed cells. The reticulocyte count was 0.2 per cent, the icteric index 3.9. Initial hemolysis occurred in 0.44 per cent NaCl, and was complete in 0.30 per cent.

Two sisters and a niece aged nine months, exhibited the anomaly. One sister had 17 per cent of deformed cells, the other 27 per cent. The baby showed 26.9 per cent of the erythrocytes to be elliptical. A brother and two first cousins showed no alteration in the shape of their red cells.

CASE 2.—F. M., a negress aged twenty-six years, was admitted to the gynecologic service in March, 1928, and salpingectomy was performed. Microscopic examination of the blood showed elliptical erythrocytes occurring in the following percentages:

96.01 per cent in Hayem's solution
78.4 per cent in fixed smears

The elliptical cells were much more uniform in measurement than in the case of B. T. The largest cell measured was 11.5 by 5.5 micra, the smallest 8.6 by 4.8 micra. Average measurements for 1,000 cells in wet preparations were 9.6 by 5.4 by 1.8 micra. In some cells two pale-staining areas taken to represent biconcavities were seen one below the other placed

in the long axis of the cell. These cells resembled more closely than any others in this series the cells seen in the blood smear of a camel.

Hemoglobin values have ranged from 56 to 84 per cent (Sahli) in samples of blood taken at various times during the last five years. The erythrocyte counts have varied from



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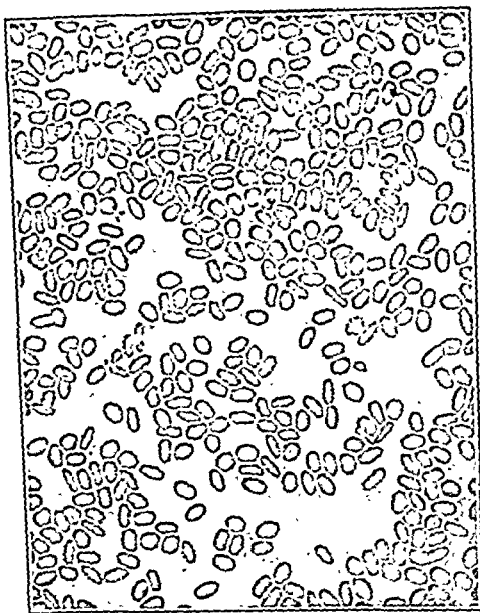


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The family of this woman proved rather difficult to examine, but the patient's three sons consented to have samples of blood withdrawn. One of them aged six years showed 17.7 per cent elliptical cells in smears, and 20 per cent in suspensions of Hayem's fluid. The other two showed no abnormalities.

CASE 3.—P. T., a negro male, aged fifty-four years, was admitted to the medical ward with lobar pneumonia in June, 1928. When the differential smear was counted a large percentage of elliptical cells were noted and the technician returned to the ward for further specimens. In the meantime, the patient had died. His relatives refused permission for autopsy and also to have specimens of their blood examined. This case is recorded merely to complete the incidence of the anomaly in this series, since confirmatory examinations could not be made.

CASE 4.—E. Mc., a negro man, aged forty-eight years, was admitted to the medical service in March, 1930, with acute alcoholism; 48 per cent of his erythrocytes were of

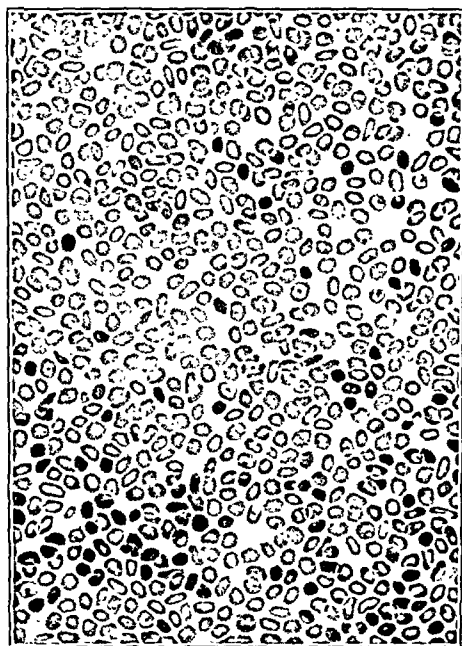


Fig. 5.

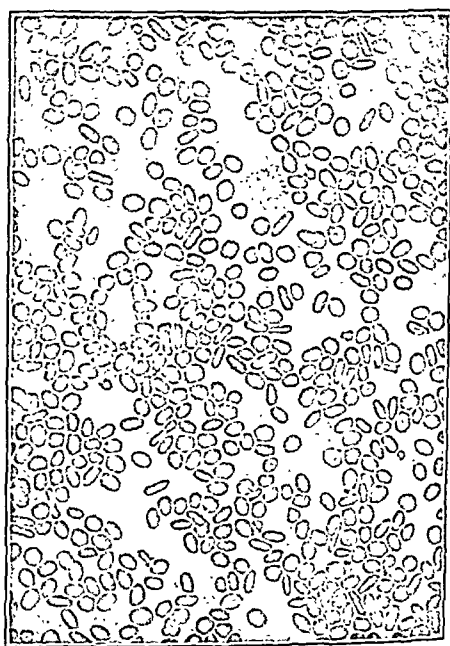


Fig. 6.

Fig. 5.—Stained film of blood of patient E. Mc. A few "double biconcave" cells can be seen.
Fig. 6.—Wet preparation of blood of E. Mc.

elliptical outline when seen in Hayem's fluid, 40 per cent were elliptical in smears. His cells were the largest noted in our series, averaging 13.3 by 5.78 by 2.89 micra (micrometer measurements in wet preparations). The round cells had an average diameter of 8 mu. Hemoglobin values have varied from 78 to 74 per cent (Sahli) red counts from 4,160,000 to 4,030,000 with a color index of 0.9. The volume index was 1.5. This is a higher value than is the average even in pernicious anemia. The cells packed to 60 per cent of the whole blood volume, whereas normal cells pack to 48 per cent of the blood volume. The volume of the elliptical cells computed from actual measurements is 193.2 c. micra. This will give some idea of their enormous size. "Fragility" test: initial hemolysis at 0.40 per cent, complete hemolysis at 0.30 per cent NaCl, reticulocytes 0.3 per cent, and icteric index 6.9.

It was possible to examine the blood from three generations of this family. Two daughters aged twenty-four and twenty-seven years, showed 15.6 and 17 per cent elliptical cells in Hayem's fluid. A granddaughter aged three years showed 10.3 per cent elliptical

cells. In examining bloods showing less than 20 per cent of the erythrocytes to be elliptical, it is rather hard to recognize the anomaly and in routine examination we would probably pass them up at best with a remark of "moderate poikilocytosis."

INVESTIGATIONS TO DETERMINE THE NATURE OF THE ANOMALY

1. The first problem that presented itself was the necessity of differentiating the elliptical phenomenon from menisocytic anemia. The two kinds of deformity are in no way comparable. In the first place, sickle cells are rarely present in large numbers in blood smears, the percentage of deformed cells increasing materially when a drop of blood is placed under a thin cover slip and an interval of several hours allowed to elapse. The percentage of elliptical cells when examined in wet preparation after a lapse of several days remains the same. Again when a drop of blood from a "sickler" is placed between a slide and cover slip and fixed in formalin by the time fixation is complete, practically every cell is deformed. Cells from elliptical cell patients show no material change in size or shape after formalin fixation. Sickle cell anemia is a disease characterized by periods of exacerbation when blood destruction is very evident. There is urobilinuria, increase of bile pigments in the serum. Examination of urine from the three elliptical cell patients on whom it was possible to do extensive work showed no urobilin. While the icteric index of one of these patients (F. M.) was 9, well within the range of latent icterus, the quantitative van den Bergh reaction gave an amount of serum bilirubin too small to estimate colorimetrically. Again sickle cell cases show at periods remarkable activity of the hematopoietic system, as indicated by enormously increased reticulocyte counts. None of these cases showed any increase in reticulocytes, and it is of note that in the literature reticulocytes were increased only in those subjects who showed a concurrent anemia. Finally, none of the clinical evidences of sickle cell anemia were present. It seems conclusive to us, therefore, that this anomaly is in no way connected with sickle cell anemia, and its causal relationship to any other anemia, or to any disease, has never been definitely established.

Our next problem was a study of the external factors which might influence the shape of these elliptical cells. Many observers have noted that various physical forces, such as pressure on the cover slip, washing repeatedly in isotonic saline solution, and increase in temperature diminished the percentage of elliptical cells, or even caused them to disappear entirely. We have never observed any change in the shape of the elliptical cells due to any of these factors. Wet preparations sealed with paraffin and observed over a period of six weeks showed no evidence of "rounding up," either when kept at room temperature, in the ice box, or in the incubator. The only change in the incubator preparations was a more rapid hemolysis than in the preparations kept at lower temperatures. Cells washed six times in isotonic saline showed no increase in the percentage of round forms by actual counts.

We tried to determine the cause of the smaller number of ellipses occurring in the smears than in the wet preparations. We found this in all but one of our cases (B. T.). This has been found also by most observers, notable exceptions being Hunter and Adams¹² who found about 10 per cent less de-

formity under No. 0 cover slips, than in stained smears. Günther¹¹ has offered a rather ingenious explanation for this "rounding up" of ellipses when they are spread out, but to me it is not entirely conclusive. I have examined smears of camel's blood, kindly furnished me by Dr. Charles Noback of the New York Zoological Gardens. At the margins of these smears, and in areas where the cells are deformed by pressure from other cells, an occasional round cell is seen. Any one who routinely examines large numbers of blood smears has observed that there is frequently considerable variation in the shape and size of the cells in certain areas, even with the greatest care of glassware and the most constant technic.

The influence of chemical agents on the shape of the elliptical erythrocytes was studied as follows:

A. *Hemolytic Agents*.—Hypotonic sodium chloride, saponin and sodium taurocholate were used. With the saponin and sodium taurocholate we prepared dilutions in the strengths recommended by Yeager.²² Cells taken from the tubes where hemolysis was not complete were practically all ellipsoid. The elliptical cells appear, therefore, more resistant to hemolytic agents than the normally shaped cells. Cells examined from the hypotonic saline solutions appeared unusually large and full, the biconcavities have been smoothed out and the enveloping membrane appears tightly stretched over the swollen, turgid cell contents. This apparent swelling of the erythrocyte is always noted just before hemolysis in normal red blood cells.²⁴

Terry^{16, 18} has devised an ingenious experiment which offers considerable support to the view that the elliptical cells are more resistant to the action of hypotonic NaCl than the round cells. He allowed the blood to sediment and examined cells taken from the upper and lower layers of the blood. He found a greater percentage of ellipses at the bottom of the tube, and concluded that these cells were heavier. He then ran "fragility" tests on both layers of cells and found that, whereas the upper layer of cells (i.e., the round cells) gave resistance curves corresponding to the normal controls, the lower layer of cells (i.e., the ellipses) showed a much wider range, that is, hemolysis began in higher concentrations of NaCl, and was complete in lower concentrations. This type of curve is one frequently observed in menisocytic anemia. We have attempted to repeat his experiments but have not been able to get the elliptical cells to sediment. Blood taken from the lower levels, either in samples that had been centrifuged, or allowed to sediment, showed by actual counts no increase in the number of ellipses above counts taken from the topmost layers. Another explanation of Terry's findings has occurred to us. It is well known that reticulocytes and other young erythrocytes are more resistant to hypotonic saline solutions than are the older more "fragile" cells. These cells are also in all probability heavier than the more mature cells, since they contain traces of stroma and a larger amount of hemoglobin. In both of Terry's cases the reticulocytes were increased (8 per cent in one case). I would suggest that the resistance curves he obtained may be dependent on the increased numbers of immature erythrocytes in his preparations. It is of note that some of the other observers who found increased "fragilities" also found an increased number of reticulocytes and

formity under No. 0 cover slips, than in stained smears. Günther¹¹ has offered a rather ingenious explanation for this "rounding up" of ellipses when they are spread out, but to me it is not entirely conclusive. I have examined smears of camel's blood, kindly furnished me by Dr. Charles Noback of the New York Zoological Gardens. At the margins of these smears, and in areas where the cells are deformed by pressure from other cells, an occasional round cell is seen. Any one who routinely examines large numbers of blood smears has observed that there is frequently considerable variation in the shape and size of the cells in certain areas, even with the greatest care of glassware and the most constant technic.

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STUDIES IN THE SEROLOGY OF SYPHILIS*

XIII. THE USE OF THE SAME ANTIGEN FOR THE WASSERMANN REACTION AND THE AUTHOR'S FLOCCULATION TEST; AND A RECOMMENDED WASSERMANN TECHNIC

HARRY EAGLE, M.D., BALTIMORE, MD.

IN A previous paper of this series¹ I recommended the use of a more strongly fortified antigen in the Wassermann reaction, an alcoholic beef heart extract supersaturated with cholesterol (0.8 per cent) and sitosterol (0.6 per cent). This antigen was used in some 80,000 tests at the Johns Hopkins Hospital, and proved highly sensitive and specific. A distinct anticomplementary action of the 1:200 antigen dilution, due to the coarseness of the lipid suspension, was completely masked when one used 0.1 or 0.05 of serum in the test, in a total volume of from 0.05 to 0.06 c.c.

A flocculation test was subsequently devised² based on the use of a new sterol derived from corn germ as an adjunct to cholesterol, each sensitizer being added to a purified alcoholic beef heart extract in a concentration of 0.6 per cent. A priori, this flocculation antigen should prove equally serviceable in the Wassermann test, for the two antigens differ in no essential respect save the substitution of corn germ sterol for sitosterol. In actual practice, they have been found to yield practically identical results in a series of 2,630 Wassermann tests on approximately 400 patients with syphilis, and on approximately 1,300 patients with various diseases in a general hospital and dispensary population (Table I).

*From the Syphilis Division of the Department of Medicine, Johns Hopkins Medical School.
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anomaly have been found in all four common blood groups. Three of our hospitalized patients were in Group III (Moss). The others were not typed.

Attempts to determine the etiology of the anomaly have so far proved unsuccessful. It cannot be transmitted from individual to individual by blood transfusion.⁶ Autopsies by Terry^{16, 18} and sternal punctures and splenic punctures by a number of observers^{9, 12, 15, 17} seem to offer definite evidence that the cells are not ellipses as they are formed, but as they mature they are subjected to the influence of some as yet unknown constituent of the plasma which causes them to assume their peculiar shape in the circulating blood. They are found in the vessels of the viscera (heart, spleen, and marrow spaces) as well as in the peripheral blood. Splenectomy did not affect the shape of these erythrocytes.¹⁰

The whole question of the factors influencing the shape of the erythrocyte and its peculiar constancy, is such an unsatisfactory one that we shall probably not be able to solve this problem of the shape of these elliptical cells until the more fundamental problems surrounding the usual shape of the erythrocyte are better understood. We need to know a great deal more about hematopoiesis and the mechanism of the release of the red cells into the circulation and their ultimate fate, before we can speculate further upon these questions. The question of nomenclature has been troublesome. We quite agree with Terry and his associates that this form of poikilocytosis should not be grouped with sickle cell anemia. Ovalcytosis and ovaemia have been suggested. Neither of these terms is entirely satisfactory but none better has been suggested.

Finally it may be said that this anomaly so far as we have been able to ascertain has no relationship to disease, in particular to the hemolytic anemias. It has been found in our series to occur in about 0.04 per cent of individuals. All our cases were in negroes.

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A flocculation test was subsequently devised² based on the use of a new sterol derived from corn germ as an adjunct to cholesterol, each sensitizer being added to a purified alcoholic beef heart extract in a concentration of 0.6 per cent. A priori, this flocculation antigen should prove equally serviceable in the Wassermann test, for the two antigens differ in no essential respect save the substitution of corn germ sterol for sitosterol. In actual practice, they have been found to yield practically identical results in a series of 2,630 Wassermann tests on approximately 400 patients with syphilis, and on approximately 1,300 patients with various diseases in a general hospital and dispensary population (Table I).

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The author therefore suggests that the use of the Wassermann antigen first described by him be discontinued, and that the same antigen* be used for both the Wassermann and precipitation tests. The Wassermann technic now in use is briefly outlined below.

It should be again³ pointed out that tests with highly sensitized antigens should not be incubated for more than four to eight hours in the ice box, as even 1:100 dilutions of the antigen are significantly anticomplementary upon overnight incubation. Nevertheless, as has been shown in the preceding paper of this series,³ a four hour ice box test with a highly sensitized antigen, followed by thirty minutes' incubation at 37° C., detects a larger proportion of syphilitic sera than an eighteen hour test with an antigen so weakly fortified as to be safe for overnight use. Moreover, with the technic outlined below, highly sensitized antigens have not been found to yield false positive reactions,³ other than the unavoidable technical slip-ups encountered in any laboratory with any technic, and which in a carefully analyzed series of 26,611 tests⁴ occurred about once in every 900 tests.

An Outline of Wassermann Technic

*Antigen.**—Fifty grams of dried powdered beef heart (Difco) are extracted for fifteen minutes at room temperature or at 37° C. with 200 c.c. anesthesia ether, with frequent shaking. The mixture is filtered, and the process repeated

TABLE I

RESULTS OBTAINED WITH THE FLOCCULATION USED AS WASSERMANN ANTIGEN, IN COMPARISON WITH THE WASSERMANN ANTIGEN ORIGINALLY DESCRIBED (2,630 TESTS)

	ABSOLUTE AGREEMENT 99%			RELATIVE AGREEMENT 0.5%		RELATIVE DIS-AGREEMENT 0.4%		ABSOLUTE DIS-AGREEMENT 0.1%	
Original Wassermann antigen 1:200 dilution	+	±	-	±	+	-	±	-	+
Flocculation antigen 1:100 dilution	+	±	-	+	±	±	-	+	-
	892	106	1,606	3	10	1	9	0	3

3 times. The moist powder is washed on the filter with 100 c.c. fresh ether, dried, and extracted with 250 c.c. of 95 or 96 per cent ethyl alcohol for three or five days at 20°-37° C. The mixture is then filtered, the moist powder washed with fresh alcohol until the filtrate is colorless, and the combined filtrate and washings are made up to 250 c.c. Cholesterol (1.5 gm.) and corn germ sterol (1.5 gm.) are then added and dissolved by boiling, a final concentration of 0.6 per cent for each. The antigen is used in the Wassermann test in a 1:100 dilution, prepared by slowly pouring 99 volumes of 0.85 per cent NaCl into 1 volume of antigen.

Complement.—The pooled serum of from 3 to 7 guinea pigs, allowed to stand on the clot overnight at 0°-4° C., is used in a 1:10 dilution.

Serum.—The serum to be tested is inactivated for twenty minutes at 56° C.

Cells.—Sheep's blood is collected in citrate† under sterile precautions. Cane sugar is then added in bulk to a concentration of 2.5 per cent. Kept in the

*The antigen or the corn germ sterol can be obtained from the Digestive Ferments Co., Detroit, Michigan.

†Twenty volumes of blood into 1 volume of sterile 20 per cent Na₂-citrate.

ice box, the cells remain in good condition for at least two weeks. This use of sugar to prolong the life of the cells was first suggested by Rous and Turner,⁵ but has unaccountably failed of general adoption. Not only does the sugar postpone spontaneous laking of the cells, but most important, because their resistance to hemolysis by complement is also maintained at a high level, the incidence of doubtful (so called 1+, 2+, 3+) results is greatly diminished.

The blood is washed once or twice in 10 volumes of 0.85 per cent salt solution, and the measured cell sediment resuspended in 32 volumes of salt solution, making a 3 per cent suspension. The minimal hemolytic quantity (unit) of amboceptor is then determined (Table II), and 3 units are added to the cells in an equal volume of salt solution, making a 1½ per cent suspension of sensitized cells.

TABLE II
AMBOCEPTOR TITRATION

Cells, 3%, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	1:400	1:800	1:1,200	1:1,600	1:2,400	1:3,200	
Amboceptor, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0
0.85 NaCl, c.c.	0.4	0.4	0.4	0.4	0.4	0.4	0.6
Complement, 1:10, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Reading of hemolysis after one-half hour at 37° C. complete complete complete complete complete partial none

Two-tenths cubic centimeter of a 1:2,400 amboceptor dilution therefore represents one unit. The entire 3 per cent cell suspension is then sensitized with an equal volume of 1:800 amboceptor, making a 1½ per cent suspension of cells sensitized with three units of amboceptor.

The tests are set up as outlined in Table III, and the tubes are placed for four hours in the ice box, *followed by one-half hour in the 37° C. water-bath.*

TABLE III
THE ROUTINE SERUM WASSERMANN TEST

	SERUM CONTROL	TEST	ANTIGEN CONTROL	COMPLEMENT CONTROL
Serum, c.c.	0.1	0.1 0.05	—	— — —
Complement 1:12, c.c.	0.2	0.2 0.2	0.2	0.2 0.1 0.05
Antigen 1:100, c.c.	—	0.2 0.2	0.2	— — —
0.85% NaCl, c.c.	0.2	— —	0.2	0.4 0.5 0.5

From four to six hours at 0°-5° C., followed by one-half hour at 37° C. Then add 0.4 c.c. sensitized cells. Read results after twenty or thirty minutes at 37° C.

TABLE IV

QUANTITATIVE WASSERMANN TEST FOR THE EXACT TITRATION OF A KNOWN POSITIVE SERUM

	SERUM CONTROLS		TEST PROPER							
Serum, c.c.	0.2	0.05	0.2	0.05						
Serum 1:25, c.c.					0.4	0.2	0.1	0.05	0.025	0.0125
Complement 1:10, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Antigen 1:100, c.c.	—	—	0.2	0.2	0.2	0.2	0.2	0.2		
NaCl, 0.85%, c.c.	0.2	0.2		0.2					0.2	0.2

Complement and antigen controls as in Table III. The six tubes correspond to serum dilutions of 1, 1:4, 1:12, 1:25, 1:50, 1:100; and the reagin content of any serum may be arbitrarily expressed as the maximum dilution yielding a positive result. Thus, a reading of 44440 corresponds to a reagin titer of 50; a reading of 444420, to a titer of approximately 35, and so on.

Four-tenths cubic centimeter of the sensitized cell suspension is then added to all the tubes. The antigen controls should clear within ten minutes. The complement controls containing 0.2 and 0.1 c.c. of complement, should both hemolyze completely within one-half hour, while that containing 0.05 c.c. of complement should not, inasmuch as the three units of amboceptor automatically correspond to $2\frac{1}{2}$ or 3 units of complement. The results of the test may be read within five

TABLE V
SPINAL FLUID WASSERMANN TECHNIC

	CONTROLS		TEST PROPER								
Spinal fluid, c.c.	1.0	0.1	1.0	0.6	0.4	0.2	0.1	0.05	0.2	0.1	0.05
Spinal fluid 1:10, c.c.											
Complement 1:10, c.c.	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Antigen 1:100, c.c.			0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Four hours at 0°-5° C., followed by one-half hour at 37° C. Add 0.4 c.c. sensitized cells. Read after twenty or thirty minutes at 37° C.

minutes of the time the antigen controls have cleared, about twenty or thirty minutes in all. If neither tube of the test shows any evidence of hemolysis, the result is *positive*; if either tube shows partial hemolysis, the result is *doubtful*; if both tubes are completely hemolyzed, the result is *negative*.

For the quantitative titration of a known positive serum, the same test is carried out on a series of serum dilutions as outlined in Table IV. The spinal fluid technic is outlined in Table V. It need be carried out routinely only down to 0.05 c.c., but it is advisable to add the last three tubes with spinal fluids containing abnormally high quantities of protein.

SUMMARY

Properly diluted, the flocculation antigen described by the author has been found to be equally serviceable in the Wassermann reaction. The results are as sensitive and specific as those obtained with the highly fortified Wassermann antigen described in a previous paper of this series. It is therefore suggested that the use of the latter be discontinued, and the same antigen be used in both the Wassermann and flocculation tests. A recommended Wassermann technic is outlined.

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A PROBABLE UNUSUAL CASE OF HODGKIN'S DISEASE*

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SUCH unusual clinical and pathologic diagnostic problems were presented in the case to be reported here, that it seemed desirable to place it on record. The clinical features of the illness were those of an acute infectious process characterized by continued fever, weakness progressing to exhaustion, abdominal distention without pain, and diarrhea, all suggestive of a typhoid state, but with repeatedly negative Widal tests. The pathologic changes were characterized primarily by proliferation of reticulo-endothelium and lymphocytes, and extreme necrosis, with involvement of the liver, spleen, and abdominal lymph nodes. The pathologic interpretation of these changes was as difficult as the clinical interpretation of the syndrome. Extreme necrosis had obscured the picture almost to the point of nonrecognition. The differential identification of inflammatory granulomas such as occur in tularemia and undulant fever, from Hodgkin's granuloma or a sarcomatous neoplasm was not easily made. Finally, after prolonged study of multiple histologic preparations, we concluded that we were dealing either with an unknown granulomatous process or, as seemed more likely, an unusual manifestation of Hodgkin's disease.

REPORT OF A CASE

The patient was an unmarried white woman, aged twenty-six years, whose entire life had been spent in southern Minnesota. In childhood she and an older brother had been victims of an epidemic of catarrhal jaundice which had swept the community, but recovery had been prompt and uncomplicated. She had experienced the usual infectious diseases of childhood, and had always suffered from a "weak stomach," according to her mother. Nausea, regurgitation, or vomiting often followed excessive excitement. She had pursued an uneventful life until she was aged twenty-two years, at which time symptoms of peptic ulcer appeared. These symptoms had become so severe that six months after their onset, subacute perforation was suspected, and she was taken to a large city for observation in a hospital. Exploration had been carried out after several weeks of observation, and posterior gastroenterostomy performed. The exact nature of the lesion found by the surgeon had not been revealed to the patient or to her family. Recovery had been slow, and the patient found it necessary to observe considerable care in her diet for several years. She had been able to resume work as clerk in a postoffice, and for two years had fair health. During the spring and summer of 1931, she had become exhausted more easily than usual. In September, 1931, she had been confined to bed for several days because of general malaise and slight diarrhea. She had returned to her work, and although she felt definitely below par, was able to continue her duties for two or three weeks.

In the first week of October, gradual distention of the abdomen had begun, with the development of obstinate constipation and weakness. The patient's physician at home had been called October 11 because of persistence of these symptoms. He had been unable to ob-

*From the Section on Pathologic Anatomy and Division of Medicine, The Mayo Clinic.
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tain any more significant history than that already detailed; there had been no history of pain at any time during the recent illness. His examination had disclosed only marked tympanites and an elevation of temperature to 100° F. For a week, the patient's temperature had varied from 100° to 104° F. On two occasions it had been found to be 104° F. at 4 A.M. and 99° F. five hours later. The patient had been able to take light nourishment by mouth. Some green-colored fecal material had been obtained by repeated enemas, but the abdominal distention remained unchanged. The patient's condition gradually had become worse, and she was irrational at times. From October 18 to 21 she had passed each day, spontaneously, three or four stools of the appearance of pea soup. Her condition at this time had strongly suggested typhoid fever, a condition with which her local physician was familiar, but four successive Vidal tests were reported negative.

The patient was brought to Rochester in a state of shock October 21. Examination in the hospital revealed pallor of the extremities and face; she was bathed in cold perspiration. Her abdomen was hugely distended and tympanitic. Few, if any, intestinal sounds could be heard. The rate of her pulse was 100 beats each minute; its quality was thin and thready. Blood pressure in millimeters of mercury was 88 systolic and 66 diastolic. Respiration was shallow, and her breath had a sour odor. She was comatose, but could be aroused enough to open her eyes. The pupils were small and reacted slightly to light. The Achilles tendon reflexes were absent. The neck was not rigid, and Kernig's sign could not be elicited.

Immediate supportive measures to combat shock were instituted. Solution of acacia and glucose were administered intravenously. Studies of the blood revealed that erythrocytes numbered 4,500,000 and leucocytes 7,100 in each cubic millimeter; the concentration of hemoglobin was 68 per cent (Dare); the concentration of urea was 36 mg. and of sugar 0.09 gm. in each 100 c.c. of blood. Agglutination tests for typhoid fever, paratyphoid fever, and undulant fever gave negative results. The state of shock persisted; the pulse rate rose to 130 beats each minute, and the blood pressure decreased. Death occurred a little more than twenty-four hours after admission.

Careful questioning of the patient's immediate family revealed only three features regarding possible contacts or exposures: (1) In the year previous to her final illness, the patient had made several visits to a neighboring farm where a rabbitry was maintained. (2) There was rather close association with a pet cat. (3) The final illness appeared at the time of extensive floor varnishing in the patient's home. The fumes from the varnish were said to have made the patient very ill, but other members of the family were not thus affected. The milk supply was investigated and found to come from the local dairy, which supplied most of the milk to the village. It was said to have been pasteurized. There was no history of bites or scratches of animals, or of bites of flies or ticks. There were no endemic diseases with which the patient was known to have had contact.

Macroscopic Findings at Necropsy.—Necropsy was performed two hours after death. The body had not been embalmed. Its length was 5 feet, 4 inches (163 cm.), and the estimated weight 135 pounds (61 kg.). The abdomen was moderately distended. There was slight edema of both lower extremities.

The left pleural cavity contained 500 c.c. and the right, 350 c.c. of clear, amber-colored fluid. The lungs were normal, except for calcified subpleural tubercles which were present in the right lung. The lymph nodes of the hilum of the right lung contained caseous, fibrous, and calcified tubercles. The pericardial sac contained 75 c.c. of light, greenish-yellow, clear fluid. The heart weighed 205 gm. The free edge of the mitral valve was moderately thickened, and presented very small, rounded, firmly attached elevations such as characterize rheumatic endocarditis. The myocardium appeared to be normal.

The peritoneal cavity contained 2,000 c.c. of clear, amber-colored fluid.

The spleen weighed 390 gm. The surface presented multiple rounded, slightly elevated nodules, which varied from 1 mm. to 3 cm. in diameter. These nodular areas appeared not to differ in color from the intervening depressed areas; both were purplish. The consistency of the nodules was much increased over that of the depressed portions. The appearance and size of the nodules were similar on the sectioned surface, except that they were commonly

surrounded by a hemorrhagic zone which clearly demarcated them, and their centers were usually grayish-white. The central portions suggested zones of necrosis without softening.

The liver weighed 2,250 gm. and extended 4.5 cm. below the costal margin. Small, grayish-white, slightly elevated nodules were observed on the capsular surface. These alternated with normal-appearing capsule, or with areas of dark red mottling. The nodules varied from 1 mm. to 2 or 3 cm. in diameter. The sectioned surface presented a similar appearance (Fig. 1). Little normal parenchyma was identifiable. The nodular appearance, so clearly revealed on the capsular surface, was less prominent on the sectioned surface, due to confluence of the grayish-white areas and also to their less discrete peripheral differentiation from the surrounding parenchyma or from the hemorrhagic, mottled zones. The gallbladder, bile ducts, and hilar vessels were normal.

The rugae of the stomach were hypertrophic, and the gastric mucosa was moderately congested. A stoma resulting from posterior gastroenterostomy was present, and appeared to be in good condition. A puckered scar, representative of a healed duodenal ulcer, was found on the posterior wall of the first portion of the duodenum. The jejunal and cecal mucosa presented multiple, small, petechial hemorrhages, with a small amount of free blood in the lumen of the bowel. The remainder of the gastrointestinal tract appeared normal.

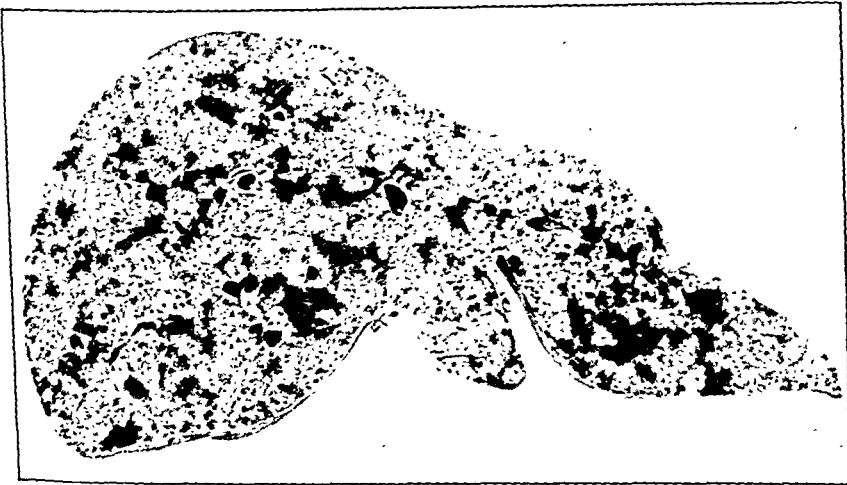


Fig. 1.—Gross appearance of the liver.

Surrounding the head of the pancreas, lesser curvature of the stomach, hepatic hilum, and aorta, retroperitoneally from the diaphragm to the bifurcation, the lymph nodes were moderately to markedly enlarged. They were firm, and grayish-white to light pink, with a glistening appearance when sectioned. Lymph nodes in other anatomic situations appeared to be normal. The pancreas, suprarenal glands, kidneys, pelvic viscera, thyroid gland, thymus, great vessels, and brain appeared normal.

Microscopic Findings at Necropsy.—Sections were prepared from tissue which had been fixed in Orth's fluid and embedded in paraffin. All were stained by hematoxylin and eosin, from which the detailed descriptions of the microscopic changes were made. Selected tissues were also stained for microorganisms by Giemsa's method, by Gram's method (Brown's modification), by Loeffler's methylene blue, by carbolfuchsin and methylene-blue acid-fast stain, and by silver impregnations (de Galantha method) for the demonstration of spirochetes.

The most striking feature of the hepatic lesion was extreme necrosis of the parenchyma (Fig. 2A). The necrosis apparently involved from half to two-thirds of the entire hepatic substance. This varied in degree in the various regions, from complete destruction of hepatic parenchyma to preservation of groups of cells surrounding the portal spaces, or to the preservation of larger units up to entire lobules or groups of lobules. The zones of necrosis were sharply demarcated from the preserved hepatic tissue, and in the necrotic zones usually no

trace of hepatic cells existed, they apparently having rapidly undergone autolysis, leaving dust-like chromatin particles in the necrotic regions. Intact hepatic units were never found in the central lobular zones to the exclusion of other parts of the lobule. The portions of the liver which were sharply demarcated from the necrotic fields revealed no retrogressive changes. The hepatic reticulum was not involved by the necrosis; the sinusoidal structures were still intact and engorged with blood. Hemorrhage, sometimes present in the necrotic portions, was caused not only by intense sinusoidal congestion but also by breaking down of some sinusoidal membranes or by engorgement of the central lobular vascular bed. Occasional proliferation of reticuloendothelium was observed. Aggregations of cells were identified, sometimes about the portal spaces or as isolated groups of cells near the periphery of the necrotic zones (Fig. 2*B*). In these cellular collections lymphocytes predominated, but large mononuclear leucocytes and endothelial cells were also found (Fig. 3*A*). Some of the endothelial cells and mononuclear leucocytes were giant forms, with either a single, large, clear nucleus, or with several small hyperchromatic nuclei. They had frequently phagocytosed lymphocytes or erythrocytes. Only occasionally were collections of lymphocytes found in the areas of necrosis. The walls



Fig. 2.—*A*, Extreme necrosis of hepatic parenchyma. Hematoxylin and eosin ($\times 45$); *B*, Collections of cells in portal connective tissue and at the periphery of necrotic zone. Hematoxylin and eosin ($\times 400$).

of the portal veins, and to some extent of the hepatic arteries, often contained cells similar to those described in the focal collections. In these vessels, too, proliferation of endothelial cells was often a prominent feature. Recently formed thrombi were observed in some of the medium-sized portal veins. The bile ducts were not involved.

The splenic sinuses were dilated and intensely engorged with blood. Malpighian follicles were decreased in number, and in those which had survived the lymphocytes were fewer, as were the lymphocytes of the splenic pulp. The second feature of note in the microscopic preparations was necrosis. This appeared as distinctly circumscribed zones, varying in size and shape, of granular, eosin staining material, which were either completely devoid of cellular structure, or zones in which only the faded outlines of cells were visible. Chromatin material often appeared in the central portion as dust-like particles. Sinuses at the periphery of the necrotic zones were congested. The third feature of note was the collection of cells which surrounded the necrotic fields (Fig. 3*B*), or appeared as small foci in the surrounding regions. Lymphocytes, mononuclear leucocytes, endothelial cells, and sometimes proliferated reticulum composed these collections. As in the liver, some of the mononuclear leucocytes and endothelial

cell types were multinucleated. Lymphocytes usually predominated (Fig. 4A). In many endothelial cells and mononuclear leucocytes were phagocytosed erythrocytes, lymphocytes, and cellular detritus. Endothelial cells were commonly increased in number, in the veins, arteries, arterioles, and sinuses. These were so numerous in some instances that the lumens of vessels or sinusoids were occluded. When this condition prevailed in the arteries and veins, the vascular walls were edematous, and frequently lymphocytes and large mononuclear leucocytes

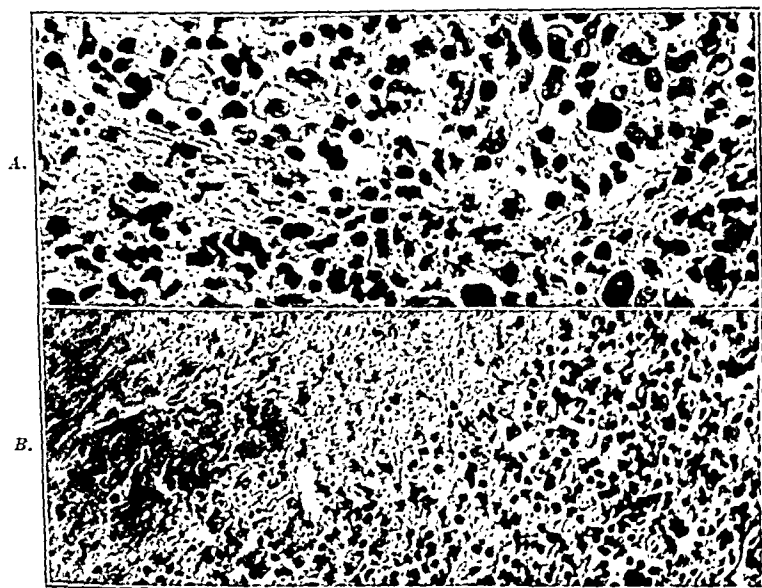


Fig. 3.—A, Lymphocytes, mononuclear leucocytes, and endothelial cells composing the cellular collections of the liver. Hematoxylin and eosin ($\times 400$). B, Cellular collection surrounding necrotic zone of spleen. The cells are chiefly lymphocytes, but mononuclear leucocytes and endothelial cells are also present. Hematoxylin and eosin ($\times 230$).

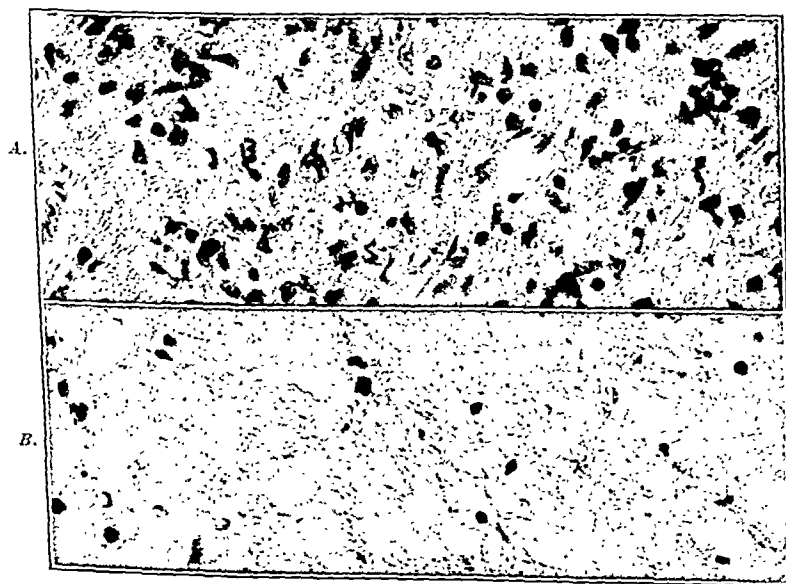


Fig. 4.—A, Fibroblasts and endothelial cells sometimes predominated in the foci of the spleen. Hematoxylin and eosin ($\times 300$). B, Necrosis with disorganization and exhaustion of lymphoid elements of nodes. The reticulum is prominently displayed. Hematoxylin and eosin ($\times 435$).

had invaded the walls. Perivascular collections of these cellular types were also present around vessels so affected. Recently formed thrombi were present in some of the veins.

The distinctive characteristics of the lesions in the gastric, hepatic, pancreatic, and retroperitoneal aortic lymph nodes, as in the liver and the spleen, were proliferative granulomatous reactions with extensive necrosis. The medullary portions of the nodes were most severely involved, where almost complete exhaustion and disorganization of the lymphoid elements

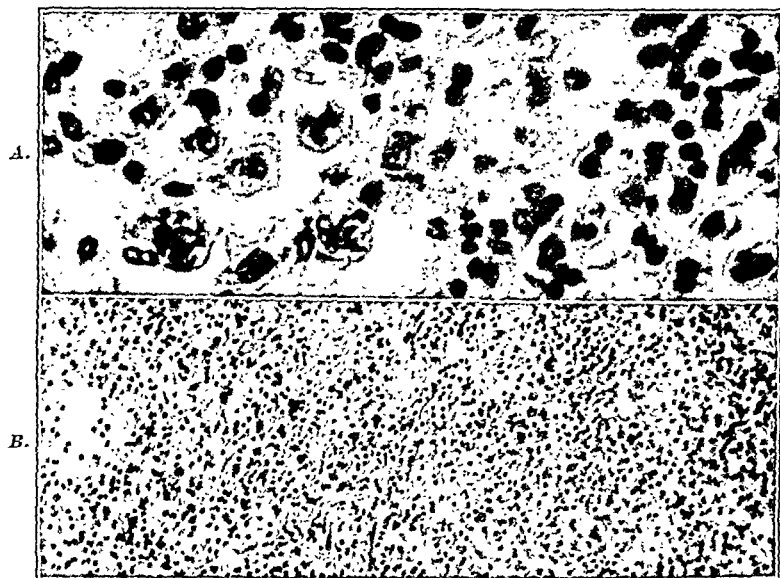


Fig. 5.—A, Dilated sinusoids of lymph node in which large endothelial cells and lymphocytes can be seen. Hematoxylin and eosin ($\times 575$). B, Local collection of lymphocytes, mononuclear leucocytes, and endothelial cells. Hematoxylin and eosin ($\times 145$).

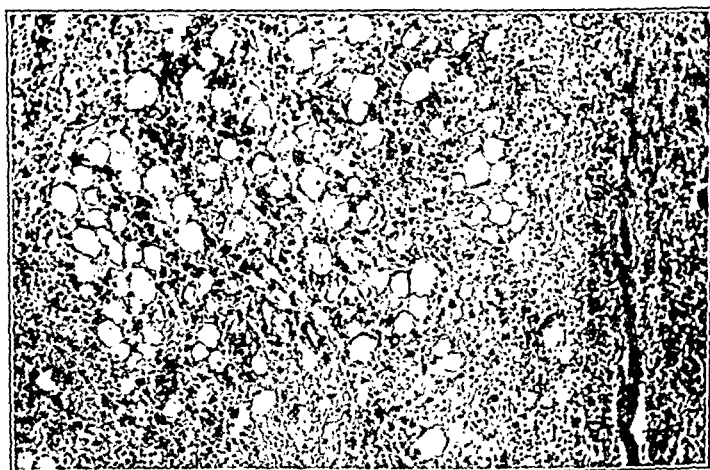


Fig. 6.—Cellular collections in the capsule of a lymph node. Hematoxylin and eosin ($\times 95$).

existed (Fig. 4B). In the cortical portion the changes were less intense, but nevertheless severe, and in this portion but few intact lymph follicles could be found. The sinusoids and reticulum were everywhere prominently displayed, due to the loss of lymphoid elements. The sinusoids were often seen as dilated spaces devoid of cells, but some contained a few cells, whereas others were engorged with cells, among which were lymphocytes, mononuclear leucocytes, erythrocytes, and large endothelial cells. The last named cells predominated (Fig. 5A).

They were distinguished usually by acidophilic cytoplasm and vesicular nuclei with a few multinucleated forms. In some, phagocytosed erythrocytes, lymphocytes, and cellular detritus were identified. Proliferated reticular cells were sometimes also revealed in the same zones. Within the medullary portions the sinuses were usually devoid of cells, or contained but few cells, whereas in the cortical portions engorgement was not infrequently observed. Focal collections of lymphocytes, large mononuclear leucocytes, and endothelial cells were also present throughout the nodes (Fig. 5B). These collections were likely to be found in any portion of the node but were usually at the periphery of the necrotic fields. Commonly, however, they were observed in the cortex, where they formed a peripheral, encircling zone from which they also extended into the capsule and surrounding tissue (Fig. 6). Within the fields of necrosis few such collections existed. Many lymph and blood vessels of the capsule contained excessive numbers of lymphocytes, mononuclear leucocytes, and endothelial cells, in addition to erythrocytes. Thrombi were sometimes present in the blood vessels. Perivascular zones of the capsule were particularly prone to reveal large increases of lymphoid cells and large mononuclear leucocytes sometimes with extravasated erythrocytes. In some of the capsules of lymph nodes, these reactions were diffuse, although rarely were they dense.

Sections of the lungs were essentially normal, but lymph nodes obtained from the hilum of the right lung disclosed tuberculous involvement. These lesions were quiescent and essentially healed.

The mitral valve was involved by a chronic, proliferative inflammatory reaction, with formation of small, hyaline-like vegetations along the line of closure.

The preparations of tissue, stained for bacteria, did not disclose convincing evidence of causation. As is well known, such preparations are sometimes difficult to interpret, and may lead to erroneous conclusions. In the stained preparations from the mitral valve and lymph nodes, small, gram-positive and gram-negative diplococci were seen. In the spleen and liver an occasional gram-positive coccoid form was revealed, but they were so few that their significance could not be emphasized. Search for other types of microorganisms by the various staining methods listed was fruitless.

Cultures in brain broth and blood agar were prepared from the blood and splenic pulp. No growth appeared. The brain broth was allowed to incubate at 37.5° C. for three weeks. Serum taken from the blood failed to agglutinate antigens prepared from *Bacterium tularensis* or *Bacterium abortus*.

COMMENT

This case presents several features in common with that reported in 1921 by Loygue and Clarion. Their patient, a soldier aged twenty years, died after an illness lasting three months, the clinical course of which was quite similar, in many respects, to that detailed for our patient. At necropsy they found the liver and spleen to be diffusely enlarged. Two large masses of lymph nodes were found, one in the gastrohepatic omentum, the other along the abdominal aorta. In addition, masses of discrete lymph nodes were found in the left axillary and cervical regions. After careful study the authors decided that tuberculosis and malignancy were definitely ruled out. Hodgkin's disease was thought to be inadmissible because of the rapid febrile course and the absence of the typical microscopic picture. They concluded that their patient died of an infectious granulomatous process of unknown etiology, but it seems likely, from their description of the microscopic findings, that they were actually dealing with a case of atypical Hodgkin's disease.

A somewhat similar case was reported by Spangenberg in 1929. The liver and spleen were enlarged and huge masses of discrete lymph nodes were found along the abdominal aorta and extending from the hilum of the spleen to the liver. Syphilis, malignancy, and tuberculosis were definitely ruled out.

Hodgkin's disease could not positively be diagnosed because of the absence of typical Sternberg cells. Diffuse lymphoid hyperplasia was the term finally selected to describe the lesion.

In our case all available clinical and pathologic data were applied to the problem of differential diagnosis. Certain infectious diseases, such as tularemia and undulant fever had to be considered. Tularemia seemed to be excluded by the negative agglutination tests obtained from the blood, postmortem. Furthermore, there was no history of contact with wild rabbits, and although the patient had visited a local rabbitry on several occasions, no case of tularemia from contact with domesticated rabbits has thus far been recorded. One case in Francis' experience resulted from the scratch of a cat, but such an occurrence was not recorded in the history of our case. Undulant fever seemed to be ruled out by virtue of negative agglutination tests made while the patient was alive and negative cultures made from the blood and spleen, postmortem. Typhoid fever and paratyphoid fever were eliminated from consideration because of the repeatedly negative agglutination tests and blood cultures and the absence of lesions in the intestines and mesenteric lymph nodes. Tuberculosis, actinomycosis, glanders, leprosy, and blastomycosis were excluded not only because of the gross and histologic characteristics of the lesions, but also because of our failure to demonstrate the specific microbes of these diseases. Rickettsia diseases such as typhus and Rocky Mountain spotted fever, so far as is known, do not occur in Minnesota and were excluded from consideration because of lack of clinical and postmortem evidence of cutaneous involvement. Furthermore, the severe changes in the spleen, liver, and lymph nodes, as revealed in our case, are not recorded for these diseases. Syphilis was considered but was excluded on the basis of absence of significant history and vascular changes and negative stains for spirochetes. The various types of leucemia were ruled out because of the absence of anemia, usually an accompaniment of acute leucemia, absence of leucocytosis, and, more especially, because the lesions in no way resembled the visceral lesions of leucemia.

A finding of questionable significance was the presence of microorganisms interpreted as diplococci in the tissues of the lymph nodes and mitral valves. The negative cultures from the blood of the heart and spleen, taken postmortem, discount the significance of such a finding. Absence of the inflammatory reactions usually considered as characteristic of conditions caused by such organisms was further evidence serving to negate the importance of this finding.

There was little evidence to support the theory that the lesions might be due to a toxic substance in the fumes of floor varnish.

A conclusion that the tissue reactions were an unusual manifestation of Hodgkin's disease or lymphosarcoma rather than one of the other diseases named or of an unknown type of infectious granuloma was finally reached as much by a process of elimination as by positive evidence. Hodgkin's disease seemed to fit into the picture better than lymphosarcoma because the pathologic process was distinctly visceral and was confined to the liver, spleen, and ab-

dominal lymph nodes. It was distinctly reticuloendothelial in its distribution and it lacked the anaplastic features one would expect in lymphosarcoma. The features which supported a diagnosis of Hodgkin's disease were: (1) proliferation of reticuloendothelial cells in and about the lesions; (2) proliferation and aggregation of lymphocytes and mononuclear leucocytes, and (3) the occurrence of giant cell types of endothelial cells and mononuclear leucocytes which resembled the Sternberg (Dorothy Reed) cells of Hodgkin's disease. The extreme necrosis, thrombosis, and perivascular collections of cells are not usually seen in Hodgkin's disease, but they do not render such a diagnosis untenable. The clinical history, although atypical, conforms in some ways with that seen in cases of visceral Hodgkin's disease. Fever is not an uncommon finding in some cases of Hodgkin's disease. The rather rapid course of the disease is unusual but in this instance may be accounted for by the extreme involvement of the liver.

In conclusion it seems best to classify our case as one of atypical Hodgkin's disease. It is apparent that our case presents many features in common with the case reported by Spangenberg and particularly with the one described by Loygue and Clarion. It would be impossible, however, to state that these three cases are clinically, etiologically and pathologically identical.

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THE PARTITION OF POTASSIUM BETWEEN THE SERUM AND CORPUSCLES IN HEALTH AND DISEASE*

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POTASSIUM IN CORPUSCLES AND SERUM

IN 1922, Kramer and Tisdall¹ found that the potassium concentration in human serum was 18 to 22 mg. per 100 c.c., while that of human red cells was about 428 mg. per 100 c.c. They showed that, except for a small amount of magnesium, potassium was the only cation in the red cells, and intimated that it balanced osmotically the cations of the plasma. Their values for normal serum have been corroborated many times, but their values for red cells have received little attention.

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The reason for the maintenance of such constant values for both red cells and serum is not apparent. But this peculiar partition suggests a physiologic balance which might be disturbed in disease. It was with this possibility in mind that a brief survey of the potassium concentration of red cells and serum in disease was undertaken. In order to facilitate this study, a new method for the determination of potassium in whole blood was devised.

METHODS

The concentration of potassium in serum was estimated by the method of Jacobs and Hoffman.² Serum total base was determined in a phosphate-free, protein-free filtrate obtained from serum by the method of Hoffman.³ In this method, coagulation by heat of the proteins of the diluted serum is effected at the iso-electric point, with the simultaneous precipitation of phosphates by the addition of ferric chloride and ammonium acetate. The results obtained are about 1 per cent too high because of slight evaporation of the hot liquid and because of the slight change in volume when the proteins are precipitated. The average value for normal serum total base, after correcting by subtracting 1 per cent, was 155.3 m.eq. per liter, and the extreme variations were 152.7 and 158.6 m.eq. per liter.

Serum chloride was determined according to Van Slyke,⁴ and calcium according to Tweedy and Koch.⁵ All other routine chemical data were taken from hospital charts. Hematocrit readings were made with Van Allen capillary pipettes,⁶ using 1.3 per cent sodium oxalate as an anticoagulant and diluting fluid.

For the whole blood potassium, a new method was devised in which the time-consuming and hazardous operation of ashing was eliminated. This was accomplished by the preparation of a suitable protein-free filtrate from whole blood by a modification of the basic ferric acetate method of deproteinization in Hoffman's³ technic for the determination of the total base of serum.

Procedure for Whole Blood Potassium.—In a 50 c.c. volumetric flask, 2 c.c. of freshly drawn whole blood were laked in about 40 c.c. of water. To this mixture were added 1 c.c. of ferric chloride reagent (A),* 1 c.c. of sodium acetate reagent (B),* and one drop of iso-amyl alcohol. The mixture was diluted with water to the mark and thoroughly shaken. About 20 c.c. were transferred to a large Pyrex test tube and heated rapidly to boiling. At the boiling point, the mixture separated into dense brownish red curds and a water-clear solution. It was filtered immediately through a double No. 40 Whatman filter paper. Filtration was very rapid. The water-clear filtrate contained no protein, no lipoids, no phosphate and no iron. It contained nothing that would interfere with the direct precipitation and determination of potassium. Its P_{H} was about 5.5.

Since the red cells contain most of the potassium, the concentration of potassium in whole blood varies with the red cell volume or is inversely proportional to the degree of anemia. For normal or nearly normal persons, 2 c.c. of filtrate contain a quantity of potassium adequate for accurate determination. If, however, there is marked anemia, that is, if the hematocrit is below 30 per cent, 4 c.c. of filtrate are required. The determination was carried out from this point on by the method employed for serum.†

*A. Ferric Chloride Reagent: Dilute 100 c.c. of 10 per cent $FeCl_3 \cdot 6H_2O$ in 0.2 N HCl to one liter with water.

B. Sodium Acetate Reagent: Dilute 100 c.c. of 15 per cent $NaC_2H_3O_2 \cdot 3H_2O$ and 20 c.c. of 1.4 N NaOH to one liter with water.

†Since the publication of our colorimetric method for the estimation of potassium, we have had occasion to make more than a thousand potassium determinations on whole blood, serum, and inorganic solutions. We have found the method consistently reliable. The technic as described will accurately determine potassium in concentrations varying from 10 to 40 mg. per 100 c.c., and can be easily adjusted for higher concentrations. The limitations of the method are those of the Kramer-Tisdall technic for the precipitation of potassium, which have been thoroughly discussed by Peters and Van Slyke.⁷ In particular, the authors have found that when potassium in concentrations of the order of 40 mg. per 100 c.c. is precipitated in the absence of sodium (which condition occurs at times in samples of ashed urine from nephrotic patients), high results are obtained. Along with Hubbard,⁸ the authors have recognized that this error can be avoided by the addition of extra sodium, especially as sodium acetate. The filtrate from whole blood, it will be noticed, contains sodium acetate, and this fact may be partly responsible for the consistent results obtained.

From the values for potassium in serum and in whole blood and from the red cell volume, the potassium content of red cells can be computed by the following formula:

$$\text{Whole Blood K} - \frac{100 - \text{Vol. per cent of cells}}{100} \times \text{Serum K} \times 100 = \frac{\text{Volume per cent of cells}}{\text{Volume per cent of cells}} \times 100 =$$

Potassium per 100 c.c. of red cells. For example: Serum K, 20.4 mg. per 100 c.c.; Whole Blood K, 201.4 mg. per 100 c.c.; Cell volume, 44.5 per cent; then,

$$\frac{201.4 - \frac{100 - 44.5}{100} \times 20.4}{44.5} \times 100 = 427.7 \text{ mg. K per 100 c.c. of red cells.}$$

The sequence used in taking samples was the following: Venous blood was drawn with-out stasis into a freshly cleaned and baked syringe. The blood was transferred into freshly cleaned and baked centrifuge tubes. Two cubic centimeters of blood were quickly transferred by means of a Folin-Ostwald pipette to the 50 c.c. volumetric flask containing about 40 c.c. of water. Then two Van Allen hematocrit tubes were prepared by first drawing up blood to the 100 mark, then by diluting it with 1.3 per cent sodium oxalate and finally by enclosing the tube in a tight-fitting, wide rubber band. All these operations had to be completed before clotting had set in. The Van Allen tubes were centrifugated for twenty minutes at 3,000 r.p.m. before reading. The blood in the centrifuge tubes was allowed to stand for fifteen minutes at 37° C. and then centrifugated. The serum separated was again centrifugated to remove all red cells. No serum was used which had any recognizable trace of hemolysis. All determinations were made in duplicate and were discarded if they did not check.

RESULTS

Table I presents data showing the adequacy of the new method for the determination of whole blood potassium. The results were less than 0.5 per cent higher than those obtained by the use of ashed whole blood. Added potassium was accurately recovered.

TABLE I
DETERMINATION OF POTASSIUM IN WHOLE BLOOD WITH ADDED POTASSIUM

	K FOUND IN ASHED WHOLE BLOOD	K FOUND IN WHOLE BLOOD THIS METHOD	K ADDED	K TOTAL	K FOUND THIS METHOD
	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.
I	192.6	192.6	18.0	210.6	211.6
			45.0	237.6	237.5
II	216.5	217.8	18.0	235.8	234.9
			45.0	262.8	260.7
III	189.0	189.2	18.0	207.2	207.5
			45.0	234.2	234.3
IV	177.3	178.1	18.0	196.1	195.2
			45.0	223.1	222.7
V	187.3	189.2	27.0	216.2	209.6
			36.0	225.2	229.8
VI	198.0	198.5	45.0	243.5	244.1
VII	192.6	192.5	36.0	228.5	228.1
VIII	178.0	178.4	45.0	223.4	223.6
IX	185.6	186.4	----	----	----
X	190.9	191.6	----	----	----
XI	191.3	191.8	----	----	----
XII	213.0	213.5	----	----	----

The distribution of potassium between serum and corpuscles was first studied in thirty normal persons. There were 17 men and 13 women, ranging in years from nineteen to thirty-nine. The determinations on women were made when they were not menstruating. The results are presented in Table II. The serum potassium averaged 19.3 mg. per 100 c.c. and the red cell potassium 422.7 mg. per 100 c.c. The individual values for the serum fell around the average within very narrow limits and corresponded with those obtained by other authors. The results for red cells fluctuated somewhat, but the standard deviation was only 3.3 per cent. There were two low values with deviations of 7 per cent and one high one with a deviation of 9.5 per cent. The values when charted for frequency (Fig. 1) do not closely approximate the theoretical probability curve, but such an approximation is hardly to be expected, since only

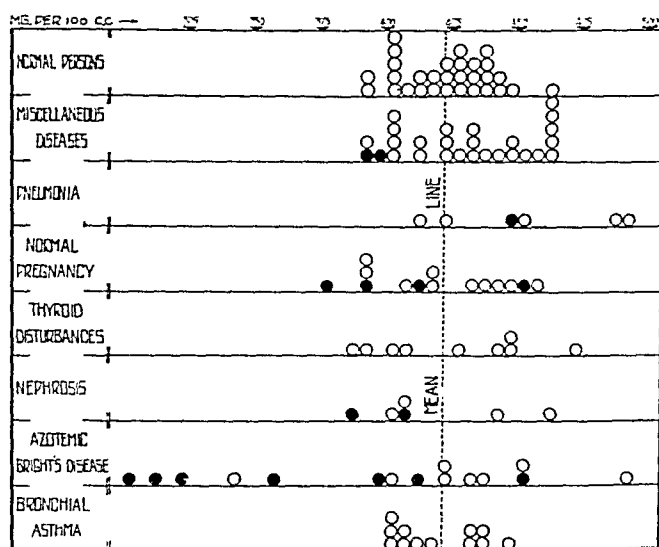


Fig. 1.—Composite frequency chart of red cell potassium concentrations in normal and diseased persons. Solid circles indicate red cell values associated with serum total base of 15.3 m.eq. per liter or less.

thirty determinations were made and since the sources of error are not only the individual physiologic variations, but also the technical errors of both the potassium determination and the hematocrit estimation. Nevertheless, the results obtained for cell potassium concentration agree closely with those obtained by Kramer and Tisdall who used a different technic.

Table III presents 27 cases of various diseases. The results obtained both for serum and red cells showed no wider deviations from the normal mean than those on normal individuals. In the cases of anemia, in spite of marked variations in whole blood potassium, cell potassium was practically always within normal limits.

Of the six cases of pneumonia reported in Table IV, two had high cell potassium concentration, which, in one case at least, was associated with a high total base concentration in the serum.

TABLE II

PARTITION OF POTASSIUM BETWEEN SERUM AND RED CELLS IN NORMAL PERSONS

CASE	SEX AND AGE	SERUM K		WHOLE BLOOD K		RED CELL K		HEMATOCRIT per cent R.B.C.
		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	
5	M 28	18.6	189.1	393.4	45.5			
12	M 32	18.8	172.4	393.4	41.0			
1	M 35	19.3	217.8	401.0	52.0			
20	M 26	18.2	185.1	401.4	43.5			
13	F 19	20.9	177.4	402.7	41.0			
11	M 29	19.0	174.5	403.9	43.0			
2	F 19	20.6	178.1	404.8	41.0			
3	F 21	18.2	189.2	406.8	44.0			
112	M 22	19.2	193.8	412.4	44.4			
103	M 35	19.3	198.5	413.0	45.5			
4	F 25	20.6	188.4	415.5	42.5			
109	M 26	19.3	192.5	417.5	43.5			
7	F 35	18.4	181.1	420.2	40.5			
108	M 33	19.0	198.2	421.7	44.5			
104	M 39	18.6	178.0	423.2	39.5			
110	F 23	18.6	188.8	425.2	41.8			
105	M 24	20.0	191.8	425.3	42.4			
15	M 32	20.4	191.8	427.7	44.5			
107	F 28	19.5	201.6	428.6	43.0			
16	M 30	20.3	193.4	431.8	44.0			
14	M 31	17.9	201.4	433.6	36.5			
8	F 26	19.0	169.6	434.9	41.5			
106	M 24	20.2	191.6	436.7	42.8			
18	F 19	19.8	198.5	438.2	38.0			
6	F 28	19.2	177.5	438.9	41.5			
17	M 27	20.4	193.4	439.5	41.6			
111	M 35	20.4	192.2	440.2	46.0			
9	F 25	19.3	213.5	440.7	36.0			
19	F 21	17.7	171.0	446.7	41.5			
10	F 31	18.0	195.8	462.8	35.5			
Average		19.3		422.7				
Stand. Deviation, %		0.73		3.3				

Fourteen determinations on pregnant women are shown in Table V. Here, too, the average values agreed closely with those for normal persons. The variations of the red cell potassium values, however, were greater than those in the normal group, four lying at or near the lower limits of normal. The average red cell potassium was found to be slightly lower than normal, but the deviations were small as compared with those reported by Rossenbeck,⁹ who found in normal pregnancy low serum potassium, low sodium, and high chloride. Spiegler,¹⁰ on the other hand, has reported high serum potassium values in normal pregnancy.

The nine cases of thyroid disturbance given in Table VI are too few to prove any definite tendencies. The results obtained showed no evidence of altered serum potassium concentration such as has been reported in thyroid disturbances.¹¹

Table VII presents the results on patients with clinical nephrosis. Three showed no signs of kidney inefficiency, while one apparently was in the nephrotic stage of chronic glomerulonephritis. In those whose edema had subsided both

TABLE III

THE PARTITION OF POTASSIUM IN A HETEROGENEOUS GROUP OF DISEASES

CASE	SEX AND AGE	PATHOLOGY	SERUM K	WHOLE BLOOD K	RED CELL K	HEMATOCRIT
			mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	per cent R.B.C.
68	M 45	Peptic ulcer, untreated	18.4	188.3	404.6	44.0
69	M 40	Carcinoma of bladder	19.2	184.3	448.0	38.5
70	M 50	Carcinoma of prostate	18.2	165.7	411.5	37.5
71	M 55	Carcinoma of stomach	16.9	175.2	393.8	42.0
72	M 40	Peptic ulcer	21.4	213.8	460.9	44.0
73	M 25	Syphilis, treated	19.9	176.7	460.8	36.0
74	F 43	Myocardial degeneration	21.4	199.8	426.8	44.0
75	M 56	Healed Pott's disease	19.4	167.0	441.0	35.0
76	M 58	Prostatic hypertrophy	18.6	164.4	423.6	36.0
77	M 26	Old gonorrheal urethritis	19.2	190.0	421.1	42.5
78	M 48	Chronic iridocyclitis	21.1	197.0	403.5	46.0
79	M 39	Urethral stricture	17.7	178.1	400.0	42.0
80	M 60	Mild diabetes mellitus	20.7	203.6	455.9	42.0
81	F 44	Persistent vomiting	17.6	142.5	396.0	33.0
82	M 63	Alcoholic pellagra	20.5	148.3	453.6	29.5
83	M 18	Toxic jaundice	19.0	182.8	410.4	42.0
84	M 34	Peptic ulcer	17.6	192.6	432.2	42.2
85	F 31	Nervous vomiting	21.1	171.0	393.8	40.2
86	M 57	Cirrhosis of the liver	21.7	150.5	448.3	30.2
87	F 22	Diarrhea	22.4	178.1	432.3	38.0
88	M 55	Myocardial degeneration with hydrothorax	17.4	198.6	462.6	40.7
89	M 25	Quiescent urticaria	19.9	196.1	422.1	43.8
102	F 25	Anxiety neurosis	19.3	185.8	433.6	40.2
58	M 58	Pernicious anemia	17.6	154.8	460.2	31.0
59	M 62	Pernicious anemia	20.6	155.5	462.7	30.5
60	M 57	Pernicious anemia	19.9	85.9	401.6	17.3
61	M 60	Secondary anemia	19.1	100.3	435.8	19.5
Average			19.6		430.7	

TABLE IV

THE PARTITION OF POTASSIUM IN PNEUMONIA

CASE	SEX AND AGE	PATHOLOGY	SERUM K	WHOLE BLOOD K	RED CELL K	HEMATOCRIT	SERUM TOTAL BASE	SERUM CHLORIDE	REMARKS
			mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	per cent R.B.C.	m.eq. per liter	m.eq. per liter	
62	M 40	Pneumonia	21.9	222.6	494.3	42.5			Fifth day
65	M 40	Pneumonia	27.1	227.6	487.6	43.5	160	109	Seventh day; lysis
63	M 44	Pneumonia	19.5	163.2	454.9	33.0			Eleventh day; no crisis
64	M 39	Pneumonia	19.8	185.1	423.4	41.0			Fifth day
66	M 29	Pneumonia	30.3	181.1	445.7	36.3	147		Eighteenth day; no crisis
67	M 50	Bronchopneumonia	18.3	171.0	414.9	38.5			Ninth day

TABLE V
 THE PARTITION OF POTASSIUM IN NORMAL PREGNANCY

CASE	AGE	MONTH OF PREGNANCY	SERUM K	WHOLE BLOOD K	RED CELL K	HEMATO-CRIT	SERUM TOTAL BASE	SERUM CHLORIDE
			mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	per cent R.B.C.	m.eq. per liter	m.eq. per liter
44	30	Second	18.6	159.5	392.3	37.7		
45	18	Fifth	19.2	175.0	458.2	35.5		
46	20	Fifth	17.6	160.7	418.5	35.7	152	
47	22	Fifth	18.2	150.0	432.7	31.8	154	
48	23	Seventh	18.6	148.0	390.5	34.8	152	107
49	30	Seventh	19.5	147.4	407.0	33.0		
50	20	Seventh	17.9	148.7	379.3	36.2	153	
51	26	Eighth	16.1	137.9	394.4	32.2	157	110
52	29	Ninth	18.8	175.2	454.5	35.2	150	106
53	35	Ninth	19.2	175.9	439.4	37.3		
54	32	Ninth	17.0	168.3	440.8	35.7		
55	35	Ninth	18.4	167.0	414.7	37.5		
56	37	Ninth	15.7	144.9	419.5	32.0		
57	26	Fourth	20.1	172.4	446.7	35.7		
Average			18.2		420.6			

 TABLE VI
 THE PARTITION OF POTASSIUM IN THYROID DISTURBANCES

CASE	SEX AND AGE	THYROID DISTURBANCES	SERUM K	WHOLE BLOOD K	RED CELL K	HEMATO-CRIT	BASAL METABOLIC RATE
			mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	per cent R.B.C.	per cent
37	M 33	Mild hypothyroid	19.3	197.9	389.5	48.5	-20
38	F 33	Postoperative myxedema	16.7	165.1	428.8	36.0	-24
39	F 45	Myxedema, treated	19.4	148.7	449.8	30.5	0
99	F 36	Hypothyroidism	20.4	167.0	444.1	34.6	-22
40	F 45	Postoperative hyperthyroid	19.6	169.6	404.2	39.0	+39
41	M 25	Simple goiter	17.8	179.6	407.5	41.2	+10
42	M 59	Hyperthyroidism	25.1	179.6	472.8	34.5	+58
43	M 40	Hyperthyroidism	18.6	193.4	394.4	46.5	+14
100	F 35	Hyperthyroidism	20.7	174.5	447.9	36.0	+32

serum and red cell potassium were essentially normal. Serum total base and chloride values were at the upper limit of normal. One patient, however, with intractable edema (Case 90) had low red cell potassium at the time the total base and chloride of the serum were low. Potassium chloride ingestion produced a simultaneous rise in both cell potassium and serum total base, associated with a slight diuresis. Later, after ammonium chloride ingestion had produced an acidosis, the low total base and low red cell potassium were unaffected by the administration of potassium citrate. The retained potassium produced only a rise in serum potassium. The significance of these results in relation to edema are further discussed in a paper by Hoffman and Post.¹²

Table VIII contains fifteen determinations in cases of azotemic Bright's disease. Six were probably cases of chronic glomerulonephritis, while the rest were for the most part cases of nephrosclerosis with or without other compli-

TABLE VII
THE PARTITION OF POTASSIUM IN NEPHROSIS

CASE	SEX AND AGE	PATHOLOGY	SERUM K mg. per 100 c.c.	WHOLE BLOOD K mg. per 100 c.c.	RED CELL K mg. per 100 c.c.	HEMATOCRIT per cent R.B.C.	SERUM TOTAL BASE m.eq. per liter	SERUM CHLORIDE m.eq. per liter	REMARKS
90	M 30	Nephrosis	18.2	145.4	388.4	34.5	153	105	Before KCl
			22.8	161.9	461.7	31.7	161	106	KCl for two weeks
			25.0	131.9	406.8	28.0	149	107	NH ₄ Cl & pot cit for 2 wk.
91	M 28	Nephrosis	22.2	195.2	407.0	45.0	157	108	No edema now
92	M 27	Nephrosis	19.3	173.8	401.7	40.4	158	111	No edema now
26	M 35	Nephrotic picture	20.0	152.6	441.0	31.5	159	113	No edema now. N.P.N. 59

eations. Four cases of azotemia associated with prostatic obstruction are included. The cases of chronic glomerulonephritis in uremia usually had low cell potassium together with low serum total base. These patients all had profound anemia, and had been vomiting enough to produce marked dehydration. The serum potassium in these cases varied in both directions from normal. The highest serum potassium concentration of all (38.4 mg. per 100 c.c.) was found in a patient (Case 27) who had been given potassium citrate for several days. In this case the red cell potassium was only 300 mg. per 100 c.c. In the other cases in this table the findings varied. Most of these patients were older than those with glomerulonephritis. They usually had a high serum potassium comparable to their nitrogenous retention, and their red cell potassium also tended to be high, particularly where there was mechanical obstruction. Their serum total base was also usually high. However, the whole group presented a number of apparently inconsistent findings, so that it was impossible to draw any rigid generalizations.

In Table IX are shown the results obtained in seven cases of allergic bronchial asthma before and after epinephrine injection. The studies were made, wherever possible, at the time of the attack and when the influence of previous injections of epinephrine had disappeared. In all cases the serum potassium values were found to be higher than normal. They were lowered within twenty minutes after the injection of ten minims of epinephrine. The lowest serum potassium of all (14.8 mg. per 100 c.c.) was found in a twenty-year-old girl ten minutes after the first injection of epinephrine for the relief of an attack of incipient asthma. This patient at the time was in a state of high nervous irritability. Unfortunately it was impossible to repeat the determination. The red cell potassium concentrations in all cases were within normal limits and were not appreciably affected by the injection of epinephrine.

TABLE VIII
THE PARTITION OF POTASSIUM IN AZOTEMIC BRIGHT'S DISEASE

CASE	SEX AND AGE	PATHOLOGY	SERUM K	WHOLE BLOOD K	RED CELL K	HEMATO- CRIT	SERUM TOTAL BASE	SERUM CHLO- RIDE	UREA N	REMARKS
			mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	per cent R.B.C.	m.eq. per liter	m.eq. per liter	mg. per 100 c.c.	
21	M 40	Chr. glom. neph.	25.5	143.1	414.6	31.0	150	104	47	
22	M 34	Chr. glom. neph.	18.4	161.3	404.6	37.0	155		33	
24	M 45	Chr. glom. neph.	16.3	82.2	397.3	17.0	147		48	Beginning uremia
25	M 35	Chr. glom. neph.	19.0	75.0	310.8	19.2	141	89	120	Uremia
27	F 27	Chr. glom. neph.	38.4	77.7	300.6	15.0	150	95	110	Uremia
28	F 37	Chr. glom. neph.	32.1	80.4	322.8	16.6	150	112	68	Uremia
29	M 85	Nephrosclerosis	31.7	131.9	420.9	25.5				N.P.N. 120 mg. per 100 c.c.
30	M 58	Nephrosclerosis	32.9	140.6	450.4	25.8		153	107	
31	M 70	Nephrosclerosis	33.3	154.9	438.6	30.0	167			N.P.N. 121 mg. per 100 c.c.
32	M 40	Nephroscler. with card. decomp.	18.2	171.0	454.6	35.0	155	108	48	
33	M 65	Hyper. pros. with retention	27.1	236.2	491.8	45.0			36	
34	M 60	Nephroscler. with hepatitis	16.4	177.4	421.9	39.7			102	
35	M 55	Hydronephrosis	29.5	95.4	343.5	21.0	169	116	184	Conn. CO ₂ 29 vols. per cent
36	M 70	Prostat. hypert.	19.4	133.6	434.7	27.5			94	Cystotomy 10 days before
35	M 67	Ca. of prostate	23.7	95.4	355.7	21.6	152	106	111	

TABLE IX

THE PARTITION OF POTASSIUM IN BRONCHIAL ASTHMA BEFORE AND AFTER EPINEPHRINE

CASE	SEX AND AGE	SERUM K	WHOLE BLOOD K	RED CELL K	HEMATO- CRIT	SERUM CA	TREATMENT WITH 10 MINIMS OF EPINEPHRINE
		mg. per 100 c.c.	mg. per 100 c.c.	mg. per 100 c.c.	per cent R.B.C.	mg. per 100 c.c.	
93	F 20	14.8	181.9	408.0	42.5	--	10 minutes after
94	F 35	23.8	167.0	410.8	37.0	9.8	
95	M 37	23.8	194.3	434.7	41.5	--	
96	M 59	26.1	197.0	401.8	45.5	--	
		24.1	194.3	418.1	43.2	9.7	Before
		19.2	184.3	403.1	43.0	9.7	12 minutes after
97	F 35	21.8	167.0	403.9	38.0	9.5	Before
		19.2	167.0	408.1	38.0	9.5	15 minutes after
98	M 40	24.4	210.6	445.6	44.2	--	Before
		21.2	206.5	438.6	44.4	--	20 minutes after
101	M 33	25.5	222.6	436.3	48.0	--	Before
		23.4	220.4	430.5	48.4	--	20 minutes after

COMMENT

Including the 30 determinations on normal adults, a total of 112 determinations of the distribution of potassium between red cells and serum have been made. It is obvious that the data are too meager to allow many generalizations. Yet certain facts are apparent. In the first place, the concentration of potassium in both red cells and serum remains remarkably constant in health and disease. In all the conditions studied, with the exception of Bright's disease and asthma, there were no wide variations from the average normal values (see Fig. 1). Even in anemia, though the whole blood potassium might be half of the normal concentration, the values for both serum and red cells were not particularly altered.

In those cases in which the red cell potassium deviated from the normal, certain other factors were present which might individually or collectively be responsible for the variations. The low red cell potassium found in most of the cases of uremia, especially in chronic glomerulonephritis, was associated with at least two other almost constant findings, low serum total base and profound secondary anemia. That there might be a correlation between serum total base and red cell potassium is suggested not only by these findings, but by a general, if not consistent, trend of low serum base with low red cell potassium. In the frequency chart (Fig. 1), the cases associated with total base of 153 m. eq. per liter or lower are found to be well on the side of low cell potassium. The attempted correlation is probably not fair, since the possible relationship was not discovered until rather late in the studies, so that total base determinations are incomplete. Nevertheless, the limited findings suggest that serum total base parallels the red cell potassium, a relationship which has a theoretical plausibility.

The degree and type of anemia may also possibly be a factor in determining the level of red cell potassium. The technical error in the determination of cell potassium increases with the degree of anemia, since the hematocrit error is increased and the concentration of potassium in the filtrate is dimin-

ished. However, it was found in general that the secondary anemia of nephritis as well as of other diseases gave rather low red cell potassium values, whereas two cases of pernicious anemia with high color index had comparatively high cell potassium. Possibly the concentration of hemoglobin determines the amount of potassium in the cells, since in all probability a good proportion of potassium is bound to hemoglobin.

Age may also be an influencing factor upon the red cell potassium levels. Twelve miscellaneous cases in which the patients were over fifty-five years old showed an average red cell potassium of 444 mg. per 100 c.c. as compared with 422 mg. per 100 c.c. in normal young adults. But the influence of age, as well as that of anemia and serum total base, on the red cell potassium level will require much further study before their importance can be properly estimated.

Serum potassium was found to be altered appreciably only in Bright's disease and in asthma. In the former disease, serum potassium was usually found to be high whenever there was marked nitrogenous retention. This rise took place often in the presence of very low red cell potassium concentrations, and suggested that in these cases there were factors preventing a rise in red cell potassium. The actual degree of rise in serum potassium in these cases was probably determined by the intake of potassium, for very high serum potassium values were found in cases that had been given potassium salts. These cases may be the ones that are more easily intoxicated by potassium medication.

In asthma, the high serum potassium and its lowering by epinephrine are in keeping with the findings in experimental anaphylaxis by numerous investigators.^{13, 14, 15} Whether there are any causal connections between the asthmatic symptoms and the level of serum potassium cannot be decided here.

SUMMARY

1. A new method for the estimation of whole blood potassium is described.
2. The partition of potassium between red cells and serum has been determined in 112 individuals.
3. In general, the levels of both serum and red cell potassium were constant both in health and disease. The normal serum potassium averaged 19.3 mg. per 100 c.c., and the red cell potassium 422.7 mg. per 100 c.c.
4. The most marked disturbances were found in Bright's disease.
5. The high serum potassium found in asthma was lowered by epinephrine.
6. Some possible relationship between red cell potassium concentration and serum total base, hemoglobin, and age are pointed out.

Most of the cases presented in this paper were patients in the Presbyterian Hospital, the Central Free Dispensary or Cook County Hospital.

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THE SCHILLING DIFFERENTIAL BLOOD COUNT IN TROPICAL DISEASES*

A STUDY OF 710 CASES WITH SPECIAL REFERENCE TO MALARIA

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WHILE the literature on the Schilling differential white blood cell count is extensive, few papers have been written of its value in tropical medicine. We thought it, therefore, worth while to report our findings of 710 cases of tropical diseases with special reference to malaria.

MATERIAL

1. Blood from 560 patients with malaria. These patients were divided into two groups, chronic and acute cases. Hemograms were studied during the different stages of the cycle, but as we found no particular change in the hemogram, only those taken during the febrile stage are charted. Hemograms were also studied during quinine therapy and after recovery.

2. Blood from 75 patients with severe secondary anemia due to intestinal parasites. These patients were divided into two groups: those who did not show intestinal ulcerations and those who presented marked intestinal pathology. Hemograms were taken on admission, during therapy, and before discharge.

3. Blood from 50 patients with leprosy, all males. These patients were divided into four groups as follows: Children with no open active lesions; children presenting lepromas in degeneration involving the bones; adults with no

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active lesions; and adults with ulcerative lesions involving the bones. Hemograms were taken from patients who had not received any therapy; from patients beginning chaulmoogra oil therapy; and from those who were under treatment of chaulmoogra oil or its esters, for a considerable length of time and who presented marked improvement.

4. Blood from 15 patients who presented tropical ulcers of three years' duration. Hemograms were studied on admission, also during calcium therapy and after two months of calcium administration, when the patients showed marked improvement.

5. Blood from 15 patients presenting mycotic disease of the skin (actinomycosis, sporotrichosis, blastomycosis). Hemograms were studied soon after admission, during iodine and calcium therapy, and before leaving the hospital or clinic.

Most of the patients were hospital cases. Those who were not, were carefully studied at the clinic. All bloods were obtained not before two hours after a meal and when the patients were well rested.

Eighty per cent of the patients were of the colored race. Due to intestinal parasitic infection which was present in 100 per cent of the colored patients and in 70 per cent of the white, most of them presented a moderate anemia and a somewhat increased eosinophile and basophile count. The hemoglobin of these patients ranged between 72 per cent and 50 per cent (Sahli). None presented a higher hemoglobin than 72 per cent and seven patients showed a hemoglobin below 50 per cent.

The hemogram was recorded after a count of 100 cells in all malaria cases and after a count of 200 cells of the bloods of all the rest. An average was taken of each group and recorded.

INTERPRETATION OF THE HEMOGRAM

In the cases studied we have been able to correlate the degree of the shift in the hemogram with the extent of the infection and gravity of the patients' condition. Like Yaguda,² John,³ McDonald,⁴ Allen,⁵ and other investigators, we have found the Schilling differential count of great diagnostic and prognostic value. In malaria, especially of the pernicious type, serial hemograms present a valuable index in the administration of quinine therapy. In the chronic cases, after quinine intake, an immediate shift to the right with a neutrophile

TABLE I
THE SCHILLING HEMOGRAM

	W.H.C.	BASOPHILES	EOSINOPHILES	MYELOCYTES	JUVENILES	BANDS	SEGMENTS	LXAMPHIOCYTES	MONOCYTES	NEUTROPHILES
Standard	7,000	1	2	0	0	4	64	23	6	68
Regenerative	10,200	0	0	2	12	35	41	9	1	90
Degenerative	3,500	0	1	0	0	25	28	40	6	53

increase takes place. In the pernicious fevers, intravenous quinine therapy brings about a quicker response and change in the hemogram than oral or intramuscular therapy. The greater and severer the infection the greater the shift to the left in the hemogram. A shift to the right with an increased white blood cell count is almost always a good prognostic index. When the patient responds

TABLE II

FIFTY CASES OF SEVERE SECONDARY ANEMIA DUE TO INTESTINAL PARASITES PRESENTING NO INTESTINAL ULCERATIONS

STANDARD	W.B.C. 7,000	B. 1	E. 2	M. 0	J. 0	B. 4	S. 64	L. 23	M. 6	N. 68
Average of 50 cases	6,500	3	9	2	0	20	40	18	8	62
Average of the same cases after successful treatment by the Rivas Method for parasites, combined with intravenous therapy of iron, arsenic, and phosphorus	7,500	1	3	0	0	9	61	20	6	70

TABLE III

TWENTY-FIVE CASES OF SEVERE SECONDARY ANEMIA DUE TO INTESTINAL PARASITES AND PRESENTING INTESTINAL ULCERATIONS

STANDARD	W.B.C. 7,000	B. 1	E. 2	M. 0	J. 0	B. 4	S. 64	L. 23	M. 6	N. 68
Average of 25 cases	11,000	2	8	1	2	21	50	12	4	74

In the above cases, the degree of the shift kept on changing as the treatment went on, and it was an excellent index in the prognosis. A shift to the right with an increase in segments, decrease in bands, and somewhat increased eosinophile count was noticed when the ulcerations began to heal. The eosinophiles and neutrophiles reached the normal level when recovery was attained. One of the patients died of intestinal perforation due to amebic ulcers, and the following hemograms were presented before death:

Two days before death	17,000	3	9	4	8	32	34	7	3	78
One day before death	8,000	3	9	4	12	40	23	7	3	78

TABLE IV

FIFTEEN CASES OF MYCOTIC DISEASE OF THE SKIN
(Actinomycosis, Sporotricosis, Blastomycosis)

STANDARD	W.B.C. 7,000	B. 1	E. 2	M. 0	J. 0	B. 4	S. 64	L. 23	M. 6	N. 68
Average of 15 cases on admission	7,000	3	9	0	2	22	42	18	4	66
Under intramuscular iodine and calcium therapy marked improvement with a shift toward the right of the hemogram was noticed	8,200	2	6	0	0	10	53	22	5	65

little to quinine, the shift to the left persists, showing a decrease in segments, and decrease in lymphocytes associated with a low white blood cell count. Our patients who died in a comatose condition, presented low white blood cell counts and marked shift to left with neutropenia. An increase of lymphocytes in the presence of a shift to the left is a favorable indication of good resistance, while

TABLE V
FIVE HUNDRED AND SIXTY CASES OF MALARIA, MIXED FORMS

STANDARD	W.B.C. 7,000	B. 1	E. 2	M. 0	J. 0	B. 4	S. 64	L. 23	M. 6	N. 68
Average of 200 chronic cases with repeated exacerbations of fever, for a period of one year or more. All presented moderate anemia	4,000	3	6	0	2	30	27	20	12	59
Average of 300 cases of acute severe infection, with high temperature. Hemograms studied during febrile stage	4,800	2	6	0	10	27	23	18	12	62
Average of 60 cases in coma with convulsions, high temperature (all cases of subtertian form)	4,500	2	3	2	12	29	23	13	15	63
Of the above group of 60, 40 responded to large doses of quinine given in glucose intravenously, and after 28 hours presented the following (average) hemogram	6,000	2	5	0	4	20	41	18	10	65
The same patients after two weeks of quinine therapy, presenting negative blood smears showed the following hemogram (average taken)	7,000	1	6	0	0	12	53	20	8	65
Twenty patients of the 60 group, who did not respond to quinine therapy and who died within one to seven days after admission, presented the following hemogram	4,000	3	4	5	19	38	6	12	13	63

TABLE VI
FIFTY CASES OF LEPROSY, 15 CHILDREN AND 35 ADULTS, ALL MALES

STANDARD	W.B.C. 7,000	B. 1	E. 2	M. 0	J. 0	B. 4	S. 64	L. 23	M. 6	N. 68
Average of 10 children; ages 7 to 14. No ulcerative lesions. Lymph positive for B. lepra	8,000	1	8	0	0	8	34	40	9	42
Average of 5 children, presenting lepromas in degeneration, involving bones of feet and hands	12,000	2	9	1	4	12	45	20	7	62
Average of 15 untreated lepra adults; no active lesions	7,500	1	5	0	0	10	56	28	8	66
These patients after the first few injections of chaulmoogra oil, presented the following hemogram	10,000	1	7	0	0	10	58	18	7	68
Average of 20 adult lepers, with active lesions involving bones of feet and hands	12,000	1	9	1	8	18	40	20	4	66
Chaulmoogra therapy in these patients, at the beginning raises the W.B.C., increases the neutrophile count and segments, and diminishes the juveniles. Hemogram after 5 days' treatment. When the patients presented marked improvement under therapy the hemogram changes toward a slight right shift	14,000	1	9	1	3	12	56	14	4	72
Average of 15 treated patients, who presented repeated negative findings in the lymph and nasal mucus during a two months' period	7,000	1	6	0	0	7	60	20	6	74

TABLE VII
FIFTEEN CASES OF TROPICAL ULCERS

STANDARD	W.B.C. 7,000	B. 1	E. 2	M. 0	J. 0	B. 4	S. 64	L. 23	M. 6	N. 68
Average of 15 cases on admission	6,300	3	7	0	4	15	46	18	7	65
After 3 intravenous calcium gluconate (Sandoz) injections, during three days, sloughing of the ulcer became noticeable and the following hemogram was recorded	9,000	2	8	0	2	15	54	16	4	70
After 24 intravenous injections of calcium (Sandoz) 10 c.c., sloughing disappeared, granulation tissue formed with marked improvement in healing. The following hemogram (average) was recorded	8,000	1	5	0	0	8	64	17	5	72

a diminished lymphocyte count, shift to left with low white blood cell count is almost always an unfavorable prognostic index.

In the anemias of parasitic origin, we found a basophilia and eosinophilia with an increase in band forms. Improvement of the patient is correlated with a decrease in eosinophiles and basophiles as well as bands with an increase in lymphocytes and segmented neutrophils. In the patients who presented severe ulcerative intestinal lesions, the disappearance of neutrophilia with an increase in lymphocytes and monocytes was always an indication of improvement, while a decrease of the white blood cell count and lymphocytes with a marked left shift was always of poor prognosis.

In mycosis, we found a slight basophilia and eosinophilia with a slight left shift in the hemogram. The patients who responded rapidly to iodine and calcium therapy showed a rapid shift to the right. Often the first few injections bring about a change in the hemogram, but should the therapy be discontinued before healing is complete, a shift to the left takes place. Recovery is indicated by a persistent slight shift to the right with a slight elevation of lymphocytes and monocytes.

In the cases of tropical ulcer, the hemograms present practically the same form as those seen in mycotic lesions.

In leprosy, we find hemograms almost identical with those found in tuberculosis. Where active lesions are present, a shift to the left is almost always present. In the treatment of the patients, the hemogram is a good indication of the response to therapy.

The progress of a patient may be very accurately followed up by frequently repeated hemograms. Like Harter and Lyons, Reznikoff,⁷ Baum⁸ and others, we consider the Shilling differential of greater value as a diagnostic and prognostic index than the Arneith or Ehrlich formula, and most readily adaptable as a routine method.

SUMMARY AND CONCLUSIONS

1. A study of the Schilling hemogram in 710 cases of tropical diseases is presented. These cases comprise 560 patients with malaria, 75 patients with severe anemia due to intestinal parasitic infestation, 50 leprosy patients, 15 cases of tropical ulcers, and 15 of mycotic disease of the skin.

2. Hemograms are reported before beginning therapy, during and at conclusion of therapy.

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LABORATORY METHODS

A RAPID COLORIMETRIC METHOD FOR MULTIPLE DETERMINATIONS OF URIC ACID*

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DETERMINATION OF URIC ACID

IN A STUDY of protein metabolism in which we are engaged, a large number of urinary uric acid determinations were called for. It was clear, at the outset, that the employment of a colorimeter would be laborious and time-consuming, chiefly because of the necessity of preparing a new standard for every sample to be analyzed. This difficulty was avoided by substituting a spectrophotometer for a colorimeter for measuring the depth of color.

The chemical reagents employed were those of Morris and Macleod.¹ The technic was modified in adapting it for use with a spectrophotometer, because in the method as it is ordinarily used there is a continuous intensification of color in both the standard and in the unknown. This is shown in Table I.

It was necessary to alter the conditions so that a maximum color, which does not change further on standing, is obtained before any readings are taken. The modification is the incubation of the uric acid together with the arsenotungstic-cyanide reagent in a total volume of 36 c.c. at 37° C. for thirty-five minutes. The solution is then cooled and made up to 50 c.c. Readings may then be taken at any time during an interval of one and one-half hours (Table III).

The advantage gained in this modification is that a large number of solutions containing unknown amounts of uric acid may be made up together. The depth of color obtained in each is then measured in the spectrophotometer and interpreted in terms of amounts of uric acid by means of an empirical curve constructed from the color intensities given by known quantities of uric acid. Having once obtained the empirical curve, it is unnecessary to make up standard solutions again for comparison with the unknown. Occasionally one standard solution is prepared to check the constancy of the reagents.

EXPERIMENTAL

The reagents and general procedure employed (with the modifications described below) were those given by Morris and Macleod.¹ The standard uric acid solution was prepared by the method of Benedict and Hitchcock.²

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A brief presentation of the principles underlying the use of a spectrophotometer for the measurement of color is given by Clark.³ We have employed a König-Martens type of instrument, with an attachment so that the angle at which the Nicol prism was set for color match when water was in train was 45° . The equation connecting differences in concentration of absorbing substance with differences in color intensity is

$$\frac{C_1}{C_2} = \frac{\log \tan^2 \theta_1}{\log \tan^2 \theta_2}$$

where θ_1 and θ_2 are the angular readings on the instrument for color match with concentrations C_1 and C_2 respectively.

One of the advantages of a spectrophotometer over a colorimeter is that independent check readings may be made at any number of desired wave lengths; any one corresponds to a reading with a colorimeter.

The absorption cell used was 20.8 mm. wide, and was covered with a glass slide while measurements were being made.

In Table I are shown the differences in the rates of development of color with three different concentrations of uric acid, when the unmodified procedure of Morris and Macleod is followed. Columns five and six show that a good proportionality exists in the first ten minutes between depth of color developed and amount of uric acid. The table also shows the necessity when a colorimeter

TABLE I

RATE OF DEVELOPMENT OF COLOR WITH VARYING AMOUNTS OF URIC ACID WITH THE UNMODIFIED TECHNIC OF MORRIS AND MACLEOD¹

Volume = 50 c.c.; $\mu = 627$

TIME MIN.	LOG TAN ² θ FOR DIFFERENT AMOUNTS OF URIC ACID			RATIO OF COLOR OF	
	0.1 MG.	0.2 MG.	0.3 MG.	$\frac{0.1 \text{ MG.}}{0.2 \text{ MG.}}$	$\frac{0.3 \text{ MG.}}{0.2 \text{ MG.}}$
5	0.62	1.25	1.8	0.50	1.44
10	0.69	1.37	1.97	0.50	1.44
15	0.74	1.44	2.06	0.51	1.43
20	0.78	1.49	2.12	0.52	1.42
25	0.81	1.54	2.18	0.53	1.41
30	0.84	1.59	2.24	0.53	1.41
40	0.89	1.67	2.33	0.53	1.40
50	0.93	1.73	2.40	0.54	1.39
60	0.97	1.79	2.46	0.54	1.39

is to be used with this technic of preparing the standard and the unknown solution at the same time.

The influence of the dilution of the reaction mixture on the intensity of color developed was not sufficiently emphasized by Morris and Macleod. Table II shows that this is an important factor; and that this must be kept constant if constant results are to be obtained. The uric acid and reagents, made up with water to the total volumes indicated in Table II were incubated at 37°C . for thirty-five minutes. They were then cooled, diluted to 50 c.c., and the intensity of color developed was measured immediately after.

It was found that if the reagents and the uric acid are kept for thirty-five minutes at 37° C., there is only a negligible further development of color after cooling to room temperature and dilution to 50 c.c. A typical result is given in Table III.

TABLE II

THE INFLUENCE OF DILUTION ON THE RATE OF DEVELOPMENT OF COLOR
Uric Acid = 0.2 mg.; Temp. = 37° C.; Time = 35 Min.; $\mu\mu$ = 607

VOLUME OF REACTION MIXTURE C.C.	LOG TAN ² θ	AMOUNT OF URIC ACID CORRESPONDING TO LOG TAN ² θ MG.
26	2.51	0.27
36	2.02	0.20
50	1.53	0.14

TABLE III

THE FURTHER DEVELOPMENT OF COLOR AT 20° C. AFTER THIRTY-FIVE MINUTES' INCUBATION AT 37° C., IN A VOLUME OF 36 C.C.; $\mu\mu$ = 607

TIME MIN.	LOG TAN ² θ	AMOUNT OF URIC ACID TO WHICH LOG TAN ² θ CORRESPONDS MG.
5	2.13	0.215
10	2.15	0.219
23	2.17	0.224
30	2.17	0.224
60	2.19	0.227
90	2.23	0.232

The following was the final procedure employed: One cubic centimeter of diluted urine, usually the original urine diluted three times, was pipetted into a 50 c.c. centrifuge tube. Thirty-eight cubic centimeters distilled water, measured with a graduate, 1 c.c. of 2.5 per cent zinc chloride, and 1 c.c. of 10 per cent sodium carbonate were then added. The solution was stirred and centrifuged. The supernatant solution was then poured off and the precipitate dissolved with 4 drops of 10 per cent HCl. This was then transferred with 30 c.c. of distilled water to a 50 c.c. volumetric flask. Five cubic centimeters of 10 per cent sodium cyanide and 1 c.c. of the arseno-18-tungstic acid solution were then added. The flask was quickly swirled, and then set away, stoppered, in a water-bath at 37° C. After thirty-five minutes it was cooled; the volume made up to 50 c.c.; and the intensity of color developed then read with the spectrophotometer.

With this procedure the data shown in Tables IV and V were obtained.

From the data in Table IV empirical calibration curves were constructed; because with the modified technic, the value of the proportionality constant relating intensity of color to the amount of uric acid is not 1. Table V gives the ratios computed from the data in Table III of the color intensity developed by different amounts of uric acid to that given by 0.2 mg. which was taken as a standard. The ratios at $\mu\mu$ 515 may be employed as correction factors when a colorimeter is used with the above procedure for development of the color; though it would be safer to construct a calibration curve.

TABLE IV

ABSORPTION AT VARIOUS WAVE LENGTHS OF SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF URIC ACID

AMOUNT OF URIC ACID MG.	ABSORPTION = $\log \tan^2 \theta$ FOR VARIOUS WAVE LENGTHS IN $\mu\mu$									
	515.5	526	537	549	561.5	575	590	607	627	670
0.05	0.38	0.41	0.44	0.48	0.53	0.58	0.63	0.71	0.77	0.82
0.10	0.63	0.68	0.74	0.82	0.90	0.99	1.10	1.21	1.34	1.45
0.15	0.81	0.89	0.98	1.08	1.20	1.33	1.48	1.64	1.81	1.97
0.20	0.97	1.08	1.19	1.31	1.46	1.62	1.80	2.00	2.19	2.38
0.25	1.14	1.26	1.39	1.53	1.72	1.91	2.12	2.34	2.59	2.79
0.30	1.33	1.47	1.61	1.79	2.00	2.23	2.48	2.76	3.06	3.29
0.35					2.22	2.47	2.76	3.08	3.36	
0.40	1.56	1.73	1.91	2.12	2.36	2.65	2.91	3.29	3.52	4.04

When only two or three urinary uric acid determinations are to be made, the unmodified procedure of Macleod and Morris is probably quicker. But, as in our case, when there is a large number of analyses the modified technic described above affords a great saving in time and labor, whether a colorimeter or spectrophotometer is used; since all the unknowns and only one standard solution may be made up together.

TABLE V

RATIOS OF COLOR INTENSITY TO AMOUNT OF URIC ACID. 0.2 MG. TAKEN AS STANDARD

AMOUNT OF URIC ACID MG.	RATIO OF URIC ACID PRESENT TO 0.20 MG.	COLOR INTENSITY FOR GIVEN AMOUNT OF URIC ACID									
		COLOR INTENSITY GIVEN BY 0.20 MG. URIC ACID AT									
		VARIOUS WAVE LENGTHS IN $\mu\mu$									
		515.5	526	537	549	561.5	575	590	607	627	670
0.05	0.25	0.39	0.38	0.37	0.36	0.36	0.36	0.35	0.35	0.35	0.35
0.10	0.50	0.65	0.63	0.62	0.63	0.62	0.61	0.61	0.61	0.61	0.61
0.15	0.75	0.84	0.83	0.82	0.83	0.82	0.82	0.82	0.82	0.83	0.83
0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.25	1.25	1.18	1.17	1.17	1.15	1.18	1.18	1.20	1.17	1.18	1.17
0.30	1.50	1.37	1.37	1.36	1.37	1.39	1.38	1.38	1.38	1.40	1.38
0.35	1.75					1.52	1.52	1.53	1.54	1.53	
0.40	2.00	1.60	1.60	1.61	1.62	1.62	1.64	1.62	1.64	1.61	1.70

TABLE VI

VALUES OBTAINED AT DIFFERENT WAVE LENGTHS IN URINARY URIC ACID DETERMINATIONS SHOWING THAT THE ABSORPTION SPECTRUM IS THE SAME FOR URINE AS FOR PURE URIC ACID

WAVE LENGTH $\mu\mu$	SAMPLE 1 MG.	SAMPLE 2 MG.	SAMPLE 3 MG.
627	0.108	0.194	0.295
607	0.108	0.194	0.297
590	0.108	0.196	0.298
575	0.108	0.195	0.299
561.5	0.109	0.195	0.297

Because approximately monochromatic light is used in the spectrophotometric readings it was necessary to know whether or not the same absorption spectrum is obtained when the uric acid is precipitated from urine as with pure uric acid; i.e., whether or not the contaminants in the zinc urate precipitate affect the color obtained. Table VI shows that the absorption spectra given by pure uric acid

and by uric acid precipitated from urine are identical. Had there been any difference the values of the different wave lengths would have exhibited a systematic variation. The agreement in the three sets of determinations in Table VI is typical of the results in more than one hundred analyses.

SUMMARY

A modification of the technic of Morris and Macleod for the determination of uric acid is described which permits much more rapid analysis of a large number of samples. If a calibration curve has been constructed previously, this technic obviates the necessity of preparing a standard solution for comparison when a spectrophotometer is used; with a colorimeter only one standard need be prepared.

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STUDIES OF THE PHYSIOLOGIC AND PATHOLOGIC CHEMISTRY OF THE SKIN*

PRELIMINARY REPORT

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MICROMETHOD FOR THE DETERMINATION OF THE SUGAR CONTENT IN THE SKIN

UNTIL recently the only functions of the skin generally considered were its protective, heat regulating, secretory, and sensory functions. There was little knowledge of its physiologic and pathologic chemistry. Wohlgemuth and his coworkers,¹ Klopstock and Buschke,² and Meleser³ were the first to demonstrate in the skin enzymes like amylase, diastase, tryptase, lipase, etc., in as high concentration as they are found in the liver. They have shown that the skin has its own specific metabolism of carbohydrate, protein, and fat, and other substances. Pillsbury⁴ found that lactic acid, which is an important product of carbohydrate metabolism, is normally present in the skin. Studies of the normal and pathologic metabolism of the skin promise to be of utmost importance in diseases of the skin as well as in other pathologic conditions. Diseases of the skin should not only be studied now from the etiologic and histologic standpoint, but consideration should also be given to the specific metabolism of the skin which may be disturbed while the metabolism of the other organs or the blood chemistry may be found to be normal.

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The work done on the enzymes has been carried out on cadavers and a few amputated limbs. Gansslen⁵ and Wohlgemuth and Scherk⁶ undertook a chemical study of the content of artificially produced blisters in man, in the belief that it would represent the chemistry of the skin *in vivo*. Urbach,⁷ who was the first to study the chemistry of the human skin *in vivo*, has demonstrated, however, a difference in the chemical values obtained from the blisters and from the skin. Together with Fantl⁸ he developed a micromethod for the determination of the sugar content in the skin. With Sieher⁹ he carried out studies on the sugar content of the skin *in vivo* under normal and pathologic conditions. They found that the fasting sugar of the human skin is normally lower than that of the blood, and that the sugar in the skin increases parallel with the sugar in the blood. In some skin diseases, as well as in latent diabetes, they found that after the ingestion of 100 gm. of glucose, the sugar in the skin increased above normal, although the blood sugar rise was within normal limits. In other cases, which according to them belong to the sympathetic endocrine group, the blood sugar curve reaches a very high level within an hour after the glucose ingestion, and rapidly falls with no abnormal increase in the height of the skin sugar curve. I found that the sugar content in normal human skin after intravenous glucose injection, and in uncontrolled diabetics is markedly elevated above the normal level. In my experiments with Abrams¹⁰ on rabbits we confirmed the findings of Folin, Trimble and Newman¹¹ that the skin sugar rises rapidly with intravenous injection of glucose and drops with the fall of the blood sugar. In addition we found that the rise in the skin sugar of the rabbit occurs as soon as ten to twenty seconds following the intravenous injection of glucose. The glucose apparently spreads rapidly by diffusion through the blood and lymph systems.

It is evident that the studies of the physiologic and pathologic chemistry of the skin should be carried out only on skin samples which represent the skin *in vivo*, as is true in the chemistry of the blood, and in order that these studies may be of practical value, micromethods should be applied. The sample of skin which is used for chemical analysis should be easily removed without any harm or great discomfort to the patient, although without anesthesia.

Urbach and Fantl's method calls for an electric Kromayer puncher and the application of two stitches or clips. The weight of the skin removed varies between 100 and 160 mg. The sugar determination on the extract is carried out by Hagedorn-Jensen method. The method proposed below is an adaptation of Folin's¹² micromethod for sugar in the blood, which is more commonly used in this country. The removal of the skin specimen and the determination of its weight is also carried out in a somewhat different manner. The test can be performed on very small pieces of skin, especially when a high sugar value is expected. It is best, however, when the skin specimen weighs in the neighborhood of 50 mg.

REMOVAL OF THE SKIN AND PROCEDURE OF THE TEST

Our aim is to obtain the skin in as normal a condition as possible. For this reason only healthy appearing skin should be removed and no local anesthetic should be used. The skin does not represent an homogenous tissue in all parts

of the body, and therefore, for the purpose of comparison, the same regions should be used in comparing different individuals, or parallel body surfaces in the same individual. I use the costovertebral region. It is free from hair, and does not require shaving. In addition, the patient is less conscious of the procedure.

The skin is cleaned with soap and water and then with alcohol about one or two hours, or if possible twelve hours before, and covered with a dry sterile dressing. The epidermis and corium of the skin is picked up with a small dry toothed forceps of rustless steel, and a piece about the size of one-quarter to one-half square centimeter is cut off quickly with a sharp, dry, sterile scalpel. The scalpel should cut through the epidermis and corium, and not touch the subcutaneous fat, if possible. There is no bleeding until after the specimen is removed. The wound is swabbed with mercurchrome and covered with a small tight dressing. The specimen is placed with the corium up into a covered Petri dish, taken immediately to the laboratory, minced,* and then weighed. In case the skin has some fat attached, which can be recognized by its yellow color, it should be removed with scissors before weighing. This is important because the sugar content of the fat is very low, and thus will lower the results of the skin sugar tests. The weight of the skin sample is determined on an analytical scale. Bang's scale is most convenient for this purpose. When the regular analytical scale is used, the skin should be weighed on a watch glass covered with another to prevent evaporation. The weight of the pair of watch glasses with and without the skin specimen is determined. The difference between the two weights gives the weight of the skin. Since the specimen is very small its weight should be recorded accurately to the tenth of a milligram. After the weight is taken, the skin is transferred into a 15 c.c. centrifuge tube containing 10 c.c. of dilute tungstic acid solution, as is used in Folin's¹² micromethod for sugar. When the weight of the skin sample is below 50 mg. and the sugar content is expected to be low, only 5 c.c. of the tungstic acid solution should be used. The skin is well stirred with a glass rod and the tube is then placed in a cool place, preferably a refrigerator, for twenty-four hours. The extraction is often completed in four hours.

From this point the test is carried out as described by Folin¹² in his improved micromethod for the determination of sugar in laked blood. For greater convenience I am giving here the full procedure.

Transfer 4 c.c. of the skin extract to a test tube graduated (with a ring all around) at 12½ and 25 c.c. Make two standards, one containing 4 c.c. of the standard sugar solution, and the other containing 2 c.c. of the standard sugar solution and 2 c.c. of distilled water in two similar tubes. To each tube add 1 c.c. of the 0.4 per cent potassium ferrieyanide solution and 1 c.c. of the cyanide carbonate solution. When the sugar content of the skin is expected to be high, 2 c.c. of the potassium ferrieyanide should be used. Heat in boiling water-bath for eight minutes, and cool in running water for from one to two minutes. Add 5 c.c. of the ferric iron solution and mix. Let stand for from one to two minutes, and then dilute with water to the 25 c.c. mark and mix. In case the color of the sample is light, dilute it

*Only nonrustable scissors and forceps should be used, since the iron of the instruments may contaminate the test.

only to 12½ c.c. and the standard to 25 c.c. In case a little foam is obscuring the surface of the solution, a couple of drops of ethyl alcohol may be added before diluting with water. For the colorimetric reading it is advisable to use Malumros' acid picrate light filter.

CALCULATION

The calculation differs from that in Folin's¹² micromethod, in that, there is a correction needed for the weight of the skin and for the modified dilutions.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \frac{100}{\text{weight of skin in mg.}} \times \text{change in dilution} \times 100 = \frac{\text{mg. sugar per}}{100 \text{ gm. skin.}}$$

A sufficient number of tests have been done by the above method to indicate its accuracy.

First, specimens taken from cadavers and from amputated limbs were studied. The skin was usually removed by the same technic as with in vivo studies. On some occasions a larger piece of skin was taken and divided into a number of smaller fractions. In Table I are given figures showing the results obtained on several specimens of skin removed from adjacent parts of the same body or amputated limb. The skin of the cadaver, as a rule, was found to have a low sugar content unless uncontrolled diabetes was present or intravenous glucose was used before death. Most of the sugar values obtained from skin samples of the same part of the body agree within 10 per cent. In Table II

TABLE I

NAME	NUMBER OF SKIN SPEC.	SKIN SUGAR								REMARKS
		MG. SUGAR PER 100 GRAMS SKIN								
E. B.	2	84	83							Amputation of leg
I. L.	2	151	143							Autopsy. Diabetic
A. B.	4	185	170	187	193					Autopsy. Died shortly after in- travenous glucose adm.
L. J.	7	92	90	94	79	95	94	92		Autopsy
J. T.	4	77	79	74	81					Amputation of leg
J. H.	3	21	20	18						Autopsy. Malignaney
	2	38	39							Amputation of leg
A. R.	5	53	54	54	58	60				Amputation of arm
I. C.	7	91	82	94	93	82	97	90		Amputation of leg
N. W.	4	59	58	66	60					Amputation of leg
J. W.	2	45	47							Autopsy
H. E.	3	80	94	78						Autopsy

are given the results obtained on in vivo removed specimens of skin compared with the results of the capillary blood sugar determined by the same method. The specimens in this table were taken from patients on the skin service about two to three hours after breakfast, except in one case of diabetic coma. On some occasions the skin specimen was divided in two pieces. Each piece was weighed, and its sugar content separately determined with quite close checks. The skin sugar in most instances was somewhat higher than the capillary blood sugar. Urbach and Sieher⁹ found that the fasting skin sugar is lower than the fasting venous blood sugar. I suppose that the higher skin sugar figures I have obtained may be explained by the fact that the skin samples were mainly taken from two to three hours after breakfast and that the return of the skin

sugar to normal is slower than that of the blood sugar. Urbach and Sicher also claim that the skin sugar is elevated in some skin diseases and that diabetes mellitus can be discovered with the aid of a skin sugar tolerance test earlier than with the blood sugar tolerance test. For practical purposes, however, I believe that a single skin sugar determination carried out from about one to three hours after a high carbohydrate meal should be sufficient. Folin and Swed-

TABLE II

NAME	CAPILLARY SUGAR IN MG.	WEIGHT OF SKIN IN MG.	SKIN SUGAR MG. SUGAR PER 100 GRAMS SKIN	TIME TEST TAKEN	DIAGNOSIS
J. C.	113	18.0	113	3 hr. after breakfast	General psoriasis
F.	98	28.4	106	Fasting	General psoriasis
M. G.	116	24.6	116		Allergy
A. D.	115	22.6	125	3 hr. after breakfast	Syphilis
M. B.	93	60.0	108	10 A.M.	Urticaria
H. L.	129	44.0	194	2½ hr. after breakfast	Diabetic
D. M.	103	30.0	131		
		19.7	134	Fasting	Pruritus vulvae
H. A.	92	39.7	123		
		33.0	130	2 hr. after breakfast	Pyodermia
A. W.	89	56.1	62		
		63.6	62	10 A.M.	Psoriasis
A. K.	113	39.3	158		
		35.0	138	Fasting	Diabetes
S. S.	770	42.5	440	During treatment,	Diabetic coma
	Venous blood	44.8	483	while in coma	

berg's¹³ micromethods for the determination of nonprotein nitrogen and uric acid in unlaked blood can be adapted for the determination of the same in the skin. Experiments are now being carried out to determine the smallest size of skin sample as well as the dilutions necessary for these determinations.

SUMMARY

Attention is called to the importance of the studies of the physiologic and pathologic chemistry of the skin.

A micromethod for the determination of the sugar content of the skin in vivo is described. Similar methods for the determination of the nonprotein nitrogen and uric acid content of the skin are suggested.

The chemistry of the skin may play an important rôle in the studies of some skin diseases and metabolic disorders.

I wish to thank Dr. Jacob Swartz for his helpful suggestions and kind permission to use the cases from the Skin Service.

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A DIRECT NESSLERIZATION METHOD FOR UREA NITROGEN IN BLOOD WHERE COMMERCIAL UREASE IS EMPLOYED*

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IN RECENT years the direct nesslerization method for urea nitrogen has been coming more and more into general use. The Karr¹ modification of it has been accepted by some hospitals and private laboratories as a regular routine procedure in place of the more cumbersome, although possibly more accurate, aeration method. In view of the increasing use of the direct nesslerization method, it seems desirable to have it further improved in order to obtain more reliable results.

The following changes seem to be especially desirable:

1. To avoid or delay the formation of turbidity on nesslerization so that an accurate reading can be taken.

2. To modify it in such a way that commercial urease can be used instead of a specially prepared urease extract. The urease manufactured by a reliable company gives uniformly accurate results, while the preparation of urease extract at a hospital, besides being time consuming, frequently results in products of varying potency. Such urease extract is sometimes a source of considerable error in the urea determination. The preparation of these extracts requires careful technic and accurate checking, which is not always possible, especially in small laboratories.

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3. To obtain a color on nesslerization which will match an ammonium sulphate standard and thus eliminate the necessity for a urea standard as employed in Karr's method.

Ammonium sulphate solutions keep indefinitely and give dependable results. Urea standards have to be renewed every month with the added disadvantage that a preservative, such as toluene, may have an undesirable effect on the color of the nesslerized urea standard.

The method here described represents an attempt to answer the above-mentioned requirements. The principle consists in incubating the Folin-Wu blood filtrate with ordinary commercial powdered urease which is later removed from solution by precipitation with an additional amount of tungstic acid; the flocculent precipitate formed is filtered off through ordinary filter paper. The resulting water-clear filtrate is then nesslerized and compared against a nesslerized standard ammonium sulphate solution.

METHOD

Reagents.—

1. *Standard Ammonium Sulphate Stock Solution*: Dissolve 0.4716 gm. of C. P. ammonium sulphate in a little water and make up to one liter; 10 c.c. of this solution contains 1 mg. of nitrogen.

2. *Nessler's Solution* (2): Introduce into a large beaker or Erlenmeyer flask, about 500 c.c. of water, add 100 gm. of C. P. sodium hydroxide. Place the flask in a pan of cold water to prevent the development of heat. Let stand until all dissolves and the solution is cool. In the meantime, introduce through a paper cone into a dry 1,000 c.c. volumetric flask, 100 gm. U.S.P. mercuric iodide (Hg_2I_2), 70 gm. C.P. potassium iodide and about 400 c.c. of distilled water. Rotate the flask to dissolve. Occasionally a few grains of mercuric iodide will remain undissolved, which does not interfere. When all has dissolved, add the sodium hydroxide solution slowly with constant rotation of the flask, and make up to 1 liter with water. Mix and transfer to a bottle with a rubber stopper. Let stand overnight, preferably in an incubator at 37 or 38° C. In the morning, a fine brown precipitate will be found on the bottom of the bottle. The supernatant fluid can be siphoned off or filtered through cotton.

This stock solution keeps indefinitely. *It has to be diluted 1:5 just before use.*

3. $\frac{1}{12}$ Normal Sulphuric Acid: It is prepared from $\frac{2}{3}$ normal H_2SO_4 by diluting the latter 8 times.

Procedure.—Prepare the Folin-Wu filtrate as usual, or, if desired, the following simplified technic can be used, which invariably gives excellent results:

For each cubic centimeter of oxalated blood, add 8 c.c. of $\frac{1}{12}$ normal H_2SO_4 , and 1 c.c. of 10 per cent Na_2WO_4 solution. Rotate the flask 2 or 3 times. Filter at once. The filtrate should be water-clear. Into an ordinary test tube of about 30 c.c. capacity, pipette 10 c.c. of Folin-Wu filtrate. Incubate with approximately 0.1 gm. Urease powder (Squibb) for fifteen minutes at 50° C. To the incubated filtrate, add 1 c.c. of distilled water, 8 c.c. of $\frac{1}{12}$ normal H_2SO_4 (or 1 c.c. of $\frac{2}{3}$ normal H_2SO_4 and 7 c.c. H_2O) and 1 c.c. of a 10 per cent solution of sodium tungstate. The total volume is 20 c.c. Mix several times by inversion. Filter off the flocculent, white precipitate through ordinary filter paper. The filtrate should be water-clear.

Into a 100 c.c. graduated cylinder, pipette 10 c.c. of this final filtrate (equivalent to 0.5 c.c. of blood). Add 3 c.c. of distilled water and 2 c.c. of dilute Nessler's solution, freshly prepared from the stock solution. Adjust to 15 c.c. mark. Mix by inversion. Compare *at once* against standard ammonium sulphate solution, which is prepared as follows: 5 c.c. of stock ammonium sulphate solution (equivalent to 0.5 mg. nitrogen) are diluted with water treated with 20 c.c. of dilute Nessler's solution and made up to 100 c.c. in a volumetric flask.

If the color of the unknown is darker than that of the standard, add more of the Nessler's solution until no more increase in color is observed. (In the case of very high urea content, 20 or 25 c.c. of Nessler's solution are required.) Dilute immediately with water to desired volume until the color approaches that of the standard. Mix and compare at once. The nesslerized solution should be perfectly clear and should remain so for several minutes. The higher the nitrogen content of solution the longer it will stay clear (three minutes to one hour). Smoke and fumes in the laboratory have been observed to accelerate the formation of turbidity.

Calculation:

$$\frac{\text{Standard Reading}}{\text{Unknown Reading}} \times \frac{\text{Unknown Dilution}}{\text{Standard Dilution}} \times \frac{\frac{1}{2} \text{ mg. N in Standard}}{\times 2 \times 100} = \frac{\text{mg. Urea N in 100 c.c. of blood}}{\text{Simplified this becomes (Standard at 20)}}$$

$$\frac{20}{\text{Unknown Reading}} \times \text{Unknown Dilution} = \text{mg. Urea N in 100 c.c. of blood}$$

RESULTS

The method described was tested out on a number of bloods as well as on control solutions. The latter was done in order to ascertain that commercial urease after being removed from solution does not leave behind some substances which will affect the accuracy of the method. Standard ammonium sulphate solutions of concentrations, from 10 to 200 mg. nitrogen per 100 c.c., served as controls. They were treated exactly as if they were bloods, namely, 1 c.c. of such solution was diluted with 10 c.c. of water, mixed with 0.1 gm. of solid urease and the latter was precipitated by addition of 8 c.c. of $\frac{1}{12}$ normal sulphuric acid and 1 c.c. of a 10 per cent solution of sodium tungstate solution as described above. The results are given in Table II.

As can be seen from the table the recovery of the nitrogen is quite good, the error not exceeding ± 3 per cent, which is the theoretical error of the direct nesslerization procedure. (The error of the colorimeter reading = 1-1.5 per cent under routine conditions. The combined error of pipetting, dilution, etc., is at least 1-1.5 per cent. The total is equal to ± 3 per cent.)

This method was next tested on a number of normal bloods, to some of which known amounts of a standard urea solution were added. The results obtained were checked against the aeration procedure. Also a number of pathologic bloods was obtained from the Coney Island Hospital, New York City, and the urea determined by the same method. The results were compared with those of the Coney Island Hospital laboratory, where Karr's direct method is used exclusively.

TABLE I

SOLUTION	CONTROL OF SUCTION MG. N. PER 100 C.C.		
	GIVEN	FOUND	ERROR* PER CENT
Standard Urea	14.0	13.9	-1
Standard Urea	14.0	13.6	-3
Standard Urea	14.0	14.4	+3
St. (NH ₄) ₂ SO ₄	15.0	14.8	-1
St. (NH ₄) ₂ SO ₄	15.0	15.3	+2
St. (NH ₄) ₂ SO ₄	25.0	25.0	0

*Error ± 3 per cent.

The aeration procedure was that recommended by Myers³ in which 2 c.c. of oxalated blood are incubated for fifteen minutes at 50° C. with Squibb's urease powder and aerated for one hour. A control was introduced into each set of 3 or 4 specimens consisting of 2 c.c. of standard ammonium sulphate

TABLE II
CONTROL EXPERIMENTS ON STANDARD AMMONIUM SULPHATE SOLUTIONS

SPECIMEN STANDARD AMMONIUM SULPHATE		NITROGEN PER 100 C.C.		ERROR Per Cent*
		GIVEN	FOUND	
		Mg.	Mg.	
Control solutions	1	10.0	9.7	- 3
Control solutions	2	20.0	20.6	+ 3
Control solutions	3	20.0	20.8	+ 4
Control solutions	4	20.0	20.2	+ 1
Control solutions	5	20.0	20.4	+ 2
Control solutions	6	30.0	30.6	+ 2
Control solutions	7	50.0	50.0	0
Control solutions	8	70.0	68.0	- 3
Control solutions	9	100.0	100.0	0
Control solutions	10	100.0	99.0	- 1
Control solutions	11	150.0	145.0	- 3
Control solutions	12	200.0	180.0	-10

*Theoretical error ± 3 .

TABLE III
COMPARISON OF PROPOSED DIRECT METHOD WITH MYERS' AERATION METHOD AND WITH KARR'S DIRECT METHOD

SPECIMEN		PROPOSED METHOD NITROGEN PER 100 C.C.	AERATION METHOD	DEVIATION
		mg.	mg.	per cent
Individual bloods				
1	J. D.	16.5	16.2	1.0
2	M. R.	17.3	16.8	3.0
3	F. K.	12.0	12.2	2.0
4	S. F.	16.4	16.5	0.5
5	J. D.	17.1	16.6	3.0
6	Ger . . .	18.4	19.2	4.0
7	H. E.	13.5	13.1	3.0
8	F. N.	13.1	13.8	5.0
9	C. I. H.	16.7	15.8	6.0
10	Wei . . .	14.2	15.1	6.0
11	Co . . .	12.0	12.2	2.0
12	J. M. C.	14.7	16.0	8.0
13	C. I. H.	45.1	46.6	3.0
14	Blood + Urea	87.3	82.2	6.0
15	Blood + Urea	80.0	83.0	3.0
16	Blood + Urea	45.8	43.7	5.0
17	Blood + Urea	41.7	40.5	3.0
18	Blood + Urea	62.5	59.7	5.0
19	Blood + Urea	98.0	106.3	7.0
20	Blood + Urea	23.7	22.4	5.0
21	Blood + Urea	35.5	35.3	1.0
22	Blood + Urea	156.0	153.0	2.0
SPECIMEN		PROPOSED METHOD	KARR'S METHOD	DEVIATION
		mg.	mg.	per cent
Individual bloods				
23	C. I. H.	26.9	27.0	0
24	C. I. H.	33.3	33.3	0
25	C. I. H.	53.3	42.1	25
26	C. I. H.	49.4	48.6	2
27	C. I. H.	129.0	125.0	2
28	C. I. H.	54.2	55.0	2
Theoretical deviation				6

solution which was equivalent to 15 mg. nitrogen in 100 c.c. It was treated exactly like the blood specimens. A urea control equivalent to 14 mg. of N in 100 c.c. was also used. This was incubated with Squibb's urease and aerated along with the bloods. The nitrogen recovery in the case of both controls was from 97 per cent to 103 per cent as Table I shows.

The data on blood determinations are given in Table III. The agreement between the method proposed and the aeration method, with a few exceptions, is under 6 per cent, which is within the theoretical tolerances.

DETAILS OF METHOD

In an attempt to prevent completely the formation of turbidity a number of other urease precipitants were tried; these included *95 per cent alcohol* of which 10 c.c. were added to 10 c.c. of Folin-Wu filtrate giving a final concentration of 50 per cent alcohol; also 5 c.c. were added giving a final concentration of 30 per cent alcohol; *trichloroacetic acid*, 300 mg.; *HNO₃ concentrated*, 1 c.c.; *HCl concentrated*, 1 c.c.; *H₂SO₄*, 10 per cent solution, 0.2 c.c.; *sulphosalicylic acid*, 3 per cent solution; *NaOH*; *1 normal*, 1 c.c.; *Na₂CO₃* saturated solution, 1.5 c.c.; *BaCl₂*, dilute solutions, varying amounts.

All of these substances except *HCl*, *H₂SO₄*, 10 per cent, and alcohol 30 per cent remove the urease completely, leaving a water-clear filtrate. On nesslerizing the latter, however, a greenish tinge and immediate turbidity were produced with alcohol 50 per cent and 30 per cent, *BaCl₂*, trichloroacetic acid, *NaOH* and *Na₂CO₃*. The 10 per cent sulphuric acid, sodium hydroxide, and sodium carbonate in the concentrations used, had a bleaching effect on color. Sulphosalicylic acid, *HCl* concentrated and *HNO₃* concentrated, prevent the formation of color altogether. It follows that tungstic acid is the best precipitant of commercial urease among those tried, because it allows a fairly stable color development on nesslerization. An attempt was made to reduce the amount of tungstic acid but was found impossible because of incomplete precipitation of urease.

Commercial urease appears to be more or less completely precipitated by a sufficient quantity of tungstic acid. At least the resulting filtrate is water-clear and on addition of another portion of tungstic acid not a trace of precipitate appears. The removal of the urease from solution causes a decided improvement in the color development on nesslerization. Although the formation of turbidity is not entirely avoided, it is delayed sufficiently to enable the technician to take an accurate reading. The nesslerized solution is obtained perfectly clear and remains so for a period of time varying from three minutes to one hour depending on the concentration of urea nitrogen. It has a good orange color and can be easily compared against an ammonium sulphate standard.

The greenish tinge is entirely absent from bloods of higher urea content. These bloods give a perfectly good match. The normal bloods, from 10 to 20 mg. per 100 c.c., do appear slightly greenish in the colorimeter, but can still be matched against the standard, the deviation from aeration procedure not exceeding 6 or 7 per cent.

In contrast to Karr's procedure, the method here proposed gives quite stable color in specimens of high urea content. Turbidity never develops immediately after addition of Nessler's solution. The color is good and perfectly clear for from about fifteen minutes to one hour. The stability of color decreases as the nitrogen content approaches the normal range, as Table IV shows.

TABLE IV
PERIODS DURING WHICH NESSLERIZED SOLUTIONS REMAIN CLEAR

N. PER 100 C.C.	DURATION OF CLEAR NESSLER'S SOLUTION
mg. 100-200	20 min. to 1 hour
50-100	10 min. to 20 min.
30- 50	5 min. to 10 min.
10- 30	3 min. to 5 min.

It is therefore advisable to nesslerize each specimen separately just before reading.

TABLE V
COMPARISON OF TAYLOR AND BLAIR DIRECT METHOD WITH MYERS' AERATION METHOD

SPECIMEN		NITROGEN PER 100 C.C.		DEVIATION	STANDARD	REMARKS
		TAYLOR AND BLAIR METHOD	AERATION METHOD			
		mg.	mg.	per cent*	mm.	
1	Normal blood	15.6	15.4	1	15	Match good
2	Normal blood	10.0	11.1	10	15	Match good
3	Normal blood	14.0	13.0	7	15	Match good
4	Normal blood	12.5	12.5	0	15	Match good
5	Normal blood	11.9	12.5	5	15	Match good
6	Normal blood	15.1	15.1	0	15	Match good
7	Normal blood	15.9	10.8	30	20	Match poor
8	Normal blood	16.9	13.2	20	20	Match poor
9	Normal blood	17.5	11.9	25	20	Match poor
10	Normal blood	17.8	14.9	20	20	Match poor
11	Normal blood	16.6	12.9	30	20	Match poor
12	Normal blood	21.8	17.5	23	20	Match poor
13	{ Normal blood	20.2	16.7	21	20	Match poor
	{ Duplicate	20.0	16.7	20	20	Match poor
14	Blood + Urea	19.7	20.3	3	20	Match good
15	Blood + Urea	24.0	23.8	1	20	Match good
16	Blood + Urea	20.8	18.3	11	20	Match good
17	Blood + Urea	22.7	19.4	15	20	Match poor
18	Blood + Urea	29.3	26.5	10	20	Match poor
19	Blood + Urea	33.0	29.2	13	20	Match poor
20	Blood + Urea	39.8	38.7	3	20	Match good
21	Blood + Urea	67.6	71.1	5	20	Match good
22	Blood + Urea	95.0	98.3	3	20	Match good
23	Blood + Urea	117.0	125.5	6	20	Match good
24	Blood + Urea	148.0	153.0	3	20	Match good
25	Urea Solution (14 mg. N. per 100 c.c.)		13.6		Percentage Recovery	97
26	Same		13.4		Percentage Recovery	96

*Theoretical deviation 6 per cent.

ADDENDA

While these experiments were in progress, an excellent direct nesslerization procedure was published by Dr. W. F. Taylor and Dr. W. M. Blair of Texas.⁴ Their method was tried out in this laboratory and was found to give good results, especially with bloods of high nitrogen content.

The nesslerized solutions in this method remain perfectly clear for about an hour or longer even in solutions of normal urea content. The match against standard ammonium sulphate is good in the case of more concentrated solutions. As to the normal bloods, they appear greenish in the colorimeter and are rather difficult to match when the standard is set at 20 mm. In our hands the error rose to about 20 or 30 per cent. Shifting the standard to 15 mm. reduces the difference in tinge between the unknown and the standard, which results in better agreement with the suction method. The results are given in Table V.

COMMENT

Since turbidity formation is completely obviated in the Taylor and Blair method, it might appear unnecessary to suggest another procedure. It was decided, however, to complete these experiments for the following reason:

1. The large amount of blood (5 c.c.) required in the method of Taylor and Blair makes it inconvenient to use in some cases especially in emergencies.
2. The special urease required for this method again calls for well-trained workers, a well-equipped laboratory, and additional time.
3. The method here presented can be completed in about thirty or thirty-five minutes and requires no special reagents. Because of its speed and simplicity it may be of use in emergency cases. It is believed to give more reliable results than Karr's procedure, because the nesslerized solution is more stable. It may, therefore, replace the latter where it is accepted as a routine procedure. This method is not intended, however, to replace the standard aeration procedure since the formation of turbidity is merely delayed but not completely obviated.

SUMMARY

A direct nesslerization procedure is proposed in which commercial urease is employed. The urease is removed from solution before nesslerization, and the color of the nesslerized solution is fairly stable so that a good reading can be taken. The stability of color increases with increased concentration of urea nitrogen in blood.

A number of urease precipitants were tried out in addition to tungstic acid. They gave, however, inferior results.

The Taylor and Blair direct method was tested and found to give satisfactory results in specimens of high urea content.

I wish to express my deepest thanks to Dr. James J. Short, Director of Laboratories at the Life Extension Institute, for permission to carry out these experiments and for numerous valuable suggestions.

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A NEW METHOD FOR THE PRODUCTION OF ANTISHEEP HEMOLYSIN*

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DURING the past ten years various methods for the production of agglutinating and hemolytic antisera have been tested in the teaching laboratory of the Department of Hygiene. Each year student workers have produced antisera by intravenous, subcutaneous, and intraperitoneal inoculations of antigens prepared in different ways but rarely have these antisera been of high titer. This has been particularly true in case of hemolysin for use in the Kolmer standard Wassermann test. Tuft, Yagle and Rogers¹ having shown that intracutaneous injection of typhoid vaccines produced the most satisfactory immunologic response, it was decided to employ this route for the administration of red blood cells or serum as suggested by Stafseth.²

Two rabbits received intracutaneous inoculations of sheep serum and two received sheep red blood cells. The serum from none of these animals produced hemolysis to any appreciable degree. One of the rabbits which had received sheep cells was then given four inoculations of cells intravenously and the other six additional intracutaneous injections of cells. Neither responded by any increase of hemolytic power. The two rabbits which had received sheep serum were given three intravenous inoculations of sheep cells five days after the last intracutaneous inoculation of sheep serum, and both produced an unusually powerful hemolysin.

In order to demonstrate whether this result was accidental, 16 rabbits were inoculated as follows: 5 intracutaneous injections of sheep serum were given at forty-eight-hour intervals, the initial dose being 0.5 c.c. and the final 2.5 c.c.; after four days three intravenous injections of 1.0 c.c. of a 10 per cent suspension of sheep red blood cells were given at forty-eight-hour intervals. Eleven other rabbits received egg albumen in place of the sheep serum in order to determine whether or not a nonspecific protein stimulation would be as effective. None of the 11 produced serum which was hemolytic in a dilution above 1-8,000 and only two reacted so actively.

Of the 16 rabbits 3 died before the last inoculation of cells was given, 2 before any cell inoculations, and one after two serum inoculations. All of these animals were emaciated and had severe diarrhea. On autopsy they showed

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extensive fibrosis of the subcutaneous tissues in the thoracic or abdominal regions. There were no significant lesions at the site of the serum inoculations and no visceral lesions. It was suggested that this was a type of anaphylactic reaction.

Of the 10 animals which survived the inoculations 7 had diarrhea and subcutaneous abscesses ventrally on the same side as the serum inoculation site. These animals were profoundly ill and all were bled to death on the fourth, fifth, or sixth day after the last inoculation. No area of fibrosis was seen in this group. Three of these animals produced serum which was hemolytic in a 1-5,000 dilution; 2 in a 1-10,000; 3 in a 1-15,000; and 2 in a 1-35,000 dilution.

It is hoped that some method of treating the serum will be evolved so that the reaction will be less severe and fewer animals will be lost. In spite of the high mortality the value of the method is apparent. In a series of 30 rabbits inoculated in 1925³ with sheep cells under varying conditions, only one responded with a serum hemolytic in a dilution above 1-10,000. Since we have been pursuing these experiments, workers in the State Bacteriological Laboratory have used the method with success though with a similar loss of animals.

CONCLUSION

The intracutaneous inoculation of rabbits with sheep serum and the subsequent intravenous injection of sheep cells was followed by the production of hemolysin of unusual power.

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3. Stowell, F. R., and Beattie, M. I.: A Comparison of Methods for the Production of Anti-sheep Hemolysin in Order to Determine the Day on Which the Highest Titer Is Obtained, *Am. J. Hyg.* 5: 1, 1925.

A SIMPLE LIGHT FILTER FOR MICROBLOOD SUGAR DETERMINATIONS*

J. W. MULL, PH.D., CLEVELAND, OHIO

IN A note on his microblood sugar method† Folin states that for satisfactory results one should use a light filter that will give a uniform field when a solution of 0.2 per cent potassium ferri cyanide set at 20 is matched against a cup of distilled water. A filter that will do this satisfactorily can easily be prepared by painting a rim of paraffin 2 to 3 mm. thick around the edges of a small section of an ordinary microscope slide. The cup so made is filled with a saturated solution of picric acid, the liquid covered with a cover slip, and the whole set on the eyepiece of the colorimeter, where it does not interfere with whatever source of light is being used.

*From the Laboratory of the Maternity Hospital.

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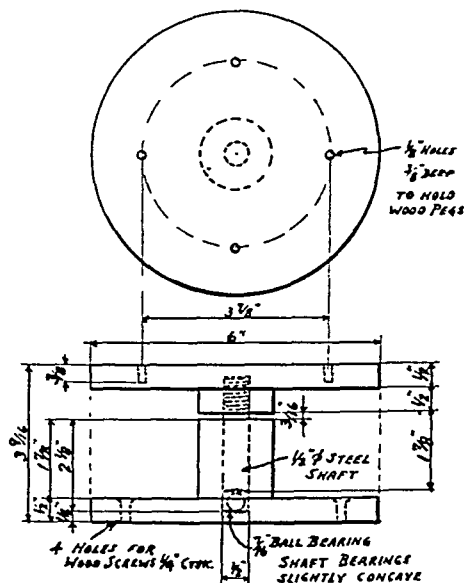
†Folin, O., and Malmros, H.: An Improved Form of Folin's Micro Method for Blood Sugar Determinations, *J. Biol. Chem.* 83: 115, 1929.

A SIMPLE ROTARY INOCULATING TABLE*

ROBB SPALDING SPRAY, PH.D., MORGANTOWN, W. VA.

MECHANICAL devices have been frequently proposed and designed to facilitate dispersion of bacteria on agar plates for the purpose of surface colony isolation. These have been, for the most part, makeshifts, or are of complicated and expensive construction, involving the use of motor or other mechanical drives.

The design here proposed has the advantage of simplicity and cheapness of construction, and can be made in any university or commercial machine shop. It consists of only three parts: namely, a cast iron turntable, base plate, and a steel ball bearing. The table runs on a steel shaft turned to an oil tight



fit in the base, and is supported on a steel ball bearing. With a touch of the finger the table spins rapidly or slowly for a minute or more, according to the impetus given.

The table is provided with four holes into which small sticks may be inserted to protrude about $\frac{1}{16}$ inch to center the dish and prevent its slipping off center if turned at high speed. Or a disk of felt may be cemented onto the table which serves the same purpose.

In use the solidified agar plates are centered on the table. A loop of culture, from broth or agar, is placed in the center. The spreader, a triangular

*From the Department of Bacteriology, Medical School, The West Virginia University.

loop or glass rod bent to a right angle, is flamed and held in the right hand. The lid is lifted with the left hand just to allow the entrance of the spreader, and the table is spun counterclockwise with the right thumb. Spiral lines or confluent inoculation may be made according to experience. With some practice excellent colony distribution is effected. The procedure is more rapid, and the results much more uniform, than are obtained by the customary stroke method.

A METABOLISM CAGE FOR RATS*

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THE desire for a more economical cage, both from the standpoint of the cost and space required, resulted in the design of the cage unit shown in Fig. 1. This unit consists of 50 cages (25 to a side) and occupies a floor space 24 by 44 inches.

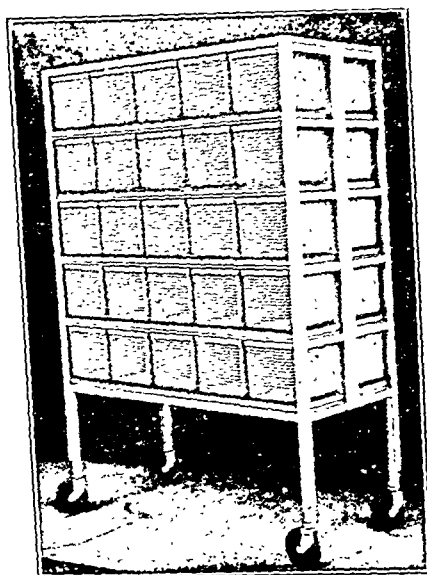


Fig. 1.

The cage proper (Fig. 2) is made in three pieces, the two sides are of galvanized iron, and the bottom and ends are of one piece of screen. The screen used is a $2\frac{1}{2}$ by $2\frac{1}{2}$ mesh, No. 063 wire, galvanized after weaving. The 24 inch width screen is cut into 8 inch pieces, and $\frac{1}{2}$ inch is turned over on a forming block after notches have been cut where the ends turn up. The side pieces are made of 24-gauge Armco galvanized iron and bent on a break as shown in the cross-section. These are then slipped onto the wire, the laps

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clined, and the corners soldered. It is best to clinch sides and solder when the cage is on a form so they will be interchangeable and slide freely into the hangers.

An opening is made in the screen by spreading the wire for inserting drinking tube, the bottle being held by a wire ring. If desired an opening

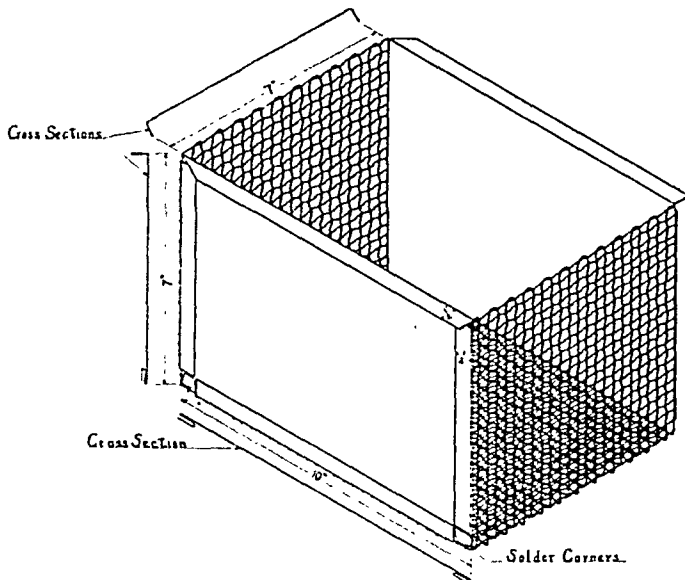


Fig. 2.

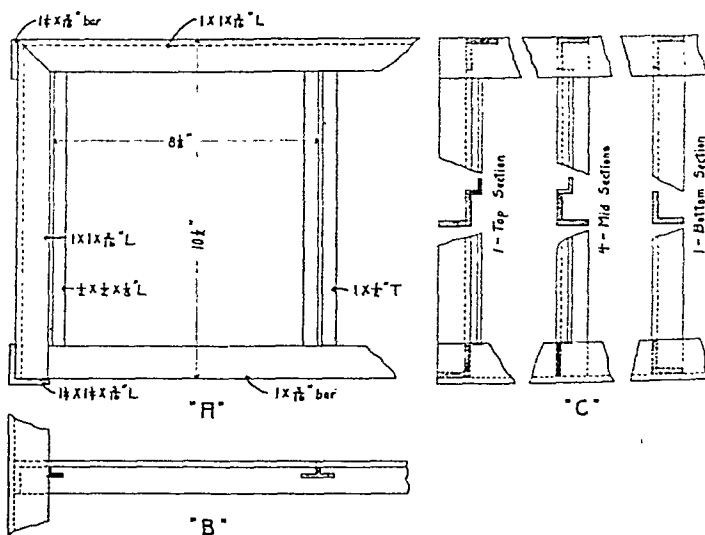


Fig. 3.

may be cut for the Joy food cup, which can be soldered on. If the wire edge does not make an even finish, strips of sheet metal can be bent on a break and soldered on as in Fig. 1.

Fig. 3 gives the dimensions and details for the construction of the stand which is made in two sections. When bolted together a sheet of galvanized iron is placed between, forming a solid back.

The $1\frac{1}{4}$ by $1\frac{1}{4}$ by $\frac{3}{16}$ inch angle and $1\frac{1}{4}$ by $\frac{3}{16}$ inch bar form the vertical supports and are welded last. Fig. 3 *A* and *B* give the detailed dimensions for the middle sections, the top (*C'*) varying from this by the substitution of an angle iron in front for the bar. The bottom section (*C'*) differs from the top by having this angle iron turned down, not requiring the angle and tees for hangers.

It was found advantageous to make a form in which to place the ends and sides for welding. The $\frac{1}{2}$ by 1 by $\frac{1}{8}$ inch angle for the hanger was then cut and spot welded. In order that the spacing be equal, templates of heavy sheet iron $8\frac{1}{8}$ inches wide were laid, and the tees for hangers were welded in place. As only a 1 by 1 by $\frac{1}{8}$ inch tee iron could be obtained, the unequal leg was cut by a torch to $\frac{1}{2}$ inch and ground smooth on an emery wheel.

The sections were then spaced $8\frac{3}{4}$ inches apart (this can be varied depending on the height of tray used to catch feces and urine), and the supporting angles and bars welded in place. As will be noted in Fig. 1, a 1 inch pipe and coupling were welded in the angle iron legs, so that the casters with 1 inch pipe thread sockets could be screwed into the couplings.

Pieces of galvanized iron were cut and laid over the top and middle sections to form the tops of the cages. On top of these, galvanized iron trays were slipped which, when filled with sawdust, catch the feces and absorb the urine.

When the iron angle framework is painted with aluminum paint, the unit has a neat and sanitary appearance.

List of material required for a unit:

- 24 ft. $1\frac{1}{4} \times 1\frac{1}{4} \times \frac{3}{16}$ inch angle
- 76 ft. $1 \times 1 \times \frac{3}{16}$ inch angle
- 15 ft. $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{8}$ inch angle
- 30 ft. $1 \times 1 \times \frac{1}{8}$ inch tee
- 17 ft. $1\frac{1}{4} \times \frac{3}{16}$ inch bar
- 30 ft. $1 \times \frac{3}{16}$ inch bar
- 200 sq. ft. 24 gauge galvanized Armeo iron (cages and trays)
- 50 sq. ft. 26 gauge galvanized iron (back and tops of cages)
- 75 sq. ft. $2\frac{1}{2} \times 2\frac{1}{2}$ galvanized screen
- 6 pounds solder
- 6 ft. 1 inch iron pipe
- 4 1 inch couplings
- 1 set casters, 5 \times 1 inch Rubberex wheels, 1 inch pipe thread socket.

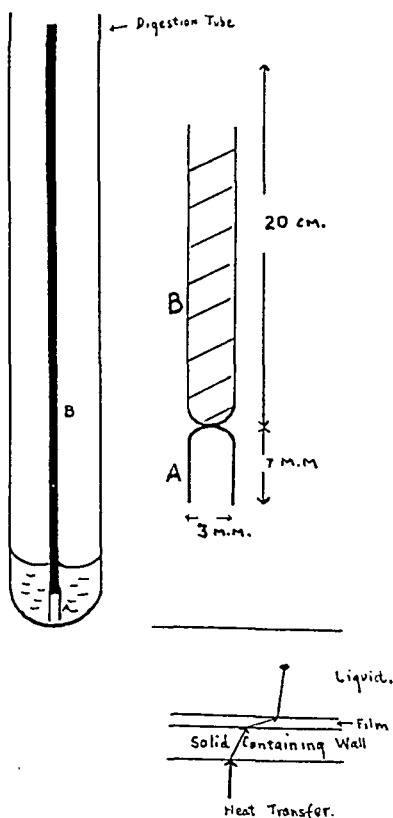
SUMMARY

A cage unit occupying minimum of space, very easy to clean, and very mobile (can be moved to balance for the weighing of rats and food, or from room to room).

A SIMPLE DEVICE TO BE USED IN CONNECTION WITH MICRO-KJELDAHL DIGESTION PROCESS*

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IT HAS been our experience that in the determination of the nonprotein nitrogen on the Folin-Wu blood filtrate by the micro-Kjeldahl method, the use of beads to prevent bumping of the liquid during the digestion has been unsatisfactory. The flame needs careful regulation and very often, in spite of



gentle heating and constant attention, the determination may be lost through bumping out of the tube.

The tube herein described has been used in the determination of the boiling points of small quantities of pure liquid. We have adapted it to the non-protein nitrogen determination. Its advantages are:

*From the Pathological Department, Bronx Hospital.

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I am indebted to Dr. Joseph Felsen, Director, for encouragement in publication of above method.

1. It prevents bumping.
2. It permits a uniform rate of boiling and makes possible the "eight minute" period for the digestion.
3. It needs no watching and many determinations may be run at the same time without fear of losing any of the mixtures.
4. It may be used repeatedly.

The device consists of a piece of Pyrex glass tubing sealed at one end, 7 mm. in length and of 3 mm. inner diameter, (*A*), attached to a Pyrex glass rod, 20 cm. in length, (*B*), and of the same diameter as (*A*). The sealed end of (*A*) is fused onto the glass rod, leaving the lower end open. The device is inserted into the bottom of the micro-Kjeldahl tube containing the blood filtrate and the "acid digestion mixture." Gentle heat is applied and after boiling has started, the digestion needs no attention until the final charring and color changes. The tube is not removed until after nesslerization; for some of the liquid goes into the open end of (*A*) and its removal at this point would introduce a source of error.

The evaporation of a liquid depends on heat transfer to the liquid through a solid containing wall. The rate of evaporation depends, among other factors, on the thickness of the film between the inner surface of the container and the liquid. This film offers the greatest resistance to heat transfer.* Superheating of the film causes sudden vaporization at the bottom of the liquid and "bumping." Smoothness and cleanliness of the solid surface are important factors in the coefficient of heat transfer.

Tube "*A*," in order to be effective, must rest in the bottom of the digestion tube. This contact compresses the film at that point and causes flashing at a lower temperature. "*A*" receives the vapor and issues it in a steady stream of small bubbles.

Thus, this device is explained by vaporization at a lower temperature, superheating of only a small portion of the liquid film, and the convection of heat by the bubbles.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PREGNANCY, Hormone Diagnosis of Viability of, Spielman, F., Goldberger, M. A., and Frank, R. T. J. A. M. A. 101: 266, 1933.

Thirty-three pregnancy cases in which missed abortion was suspected or expected were studied from the hormonal aspect in order to determine the relationship between the female sex hormone of the blood and the prepituitary hormone of the urine on the one hand, and fetal life or death on the other.

The Frank-Goldberger method was used in the blood studies, and either the Aschheim-Zondek or the Friedman test in the urine studies.

A negative female sex hormone reaction was obtained when the fetus was dead (twenty-three cases) and a positive reaction when the fetus was alive (eight cases). The results here were 100 per cent correct.

A negative pregnancy reaction was obtained when the fetus was dead in eleven cases, and ten cases gave positive reactions in spite of dead fetuses (approximately 50 per cent correct).

The female sex hormone blood determination has proved itself in our small series an absolute indicator of whether a fetus is alive or dead. The urine pregnancy test is of value only when the reaction is negative.

The presence or absence of the hormones depends on the degree of involution of the placenta and its attachment to the uterine wall.

PNEUMONIA, Significance of the Newly Classified Types of Pneumococci in Disease, Sutliff, W. D., and Finland, M. J. A. M. A. 101: 1289, 1933.

This general survey of the incidence of twenty serologically specific strains of pneumococci and of hemolytic streptococci, staphylococci and Friedlander bacilli in the pneumonia cases in a general hospital in an interepidemic period shows that each one of these organisms may cause the disease. The rare type-specific pneumococci numbered from IV to XX are consistently present in the sputum and lesions in certain cases of lobar pneumonia and bronchopneumonia. The presence of such pneumococcus strains in the sputum of patients with pneumonia is a reliable indication of their presence in the lung.

Exact etiologic diagnoses of pneumococcic pneumonia were made by the use of seventeen of the new typing serums, in a group of cases in which this has heretofore been impossible, amounting to 30 per cent of the total number of lobar pneumonia cases, and in 65.1 per cent of the relatively little understood and important group of bronchopneumonia and secondary pneumonia.

The order of frequency of the six most frequent pneumococcus types in pneumococcic lobar pneumonia is as follows: I, II, III, VIII, V and VII, making together 84.1 per cent of the cases, and the order of frequency of the ten most frequent types in pneumococcic bronchopneumonia is: III, VIII, XVIII, X, V, VII, XX, II, XI and XIV, making altogether 81.1 per cent of the cases. The six types most frequent in lobar pneumonia are also those most frequent in the whole series and have been given more attention than the others.

Certain individual characteristics of the pneumonias caused by the new pneumococcus types from IV to XX are brought out and compared with the better known Types I, II and III. The three most frequent of the newer types are V, VII and VIII. Type V makes up 5 per cent of the total of pneumococcic pneumonias or empyemas and causes either pneumonia or empyema in 96 per cent of the cases in which it is found and leads to purulent complications in 28 per cent of the pneumonia cases. Type V is associated with lobar pneumonia

rather than bronchopneumonia in only 81 per cent of the cases, in which respect it resembles most of the newer types. Its age distribution is quite irregular and without the usual preponderance in middle life. Type VII is associated with pneumonia or empyema in only 80 per cent of the cases but leads to purulent complications in 11 per cent, a rather high proportion of these pneumonia cases. Type VIII is associated with pneumonia or empyema in 90 per cent of the cases in which it is isolated and has a low mortality in lobar pneumonia, 23.8 per cent, but, owing to an average bronchopneumonia mortality of 67 per cent, has a total mortality near the average.

The experience afforded by the bacteriologic classification of this series of pneumonia cases has indicated that the pneumonias due to different serologic strains of pneumococci and due to other organisms may be regarded as separate entities. By identifying such organisms in every case, the clinician may make accurate etiologic diagnoses and prognoses, the epidemiologist may unravel the factors affecting the organism and the patient leading to the development of atypical and secondary pneumonia, and the physician may apply the large amount of information about pneumococcus type specificity to the cure of and protection from pneumococcal infections.

PNEUMONIA, Epidemiology of, Prevalence of Specific Strains in Immediate Family Contacts, Smillie, W. G. J. A. M. A. 101: 1281, 1933.

In a study of over a thousand persons, Type I and Type II pneumococci were found to be much more prevalent in the nasopharynx in immediate family contacts of cases of lobar pneumonia due to the homologous type than in the population at large.

The higher types of pneumococci—Types III to XIX, inclusive, were just as prevalent in the throat in the general population as in the family contacts of cases of pneumonia due to these specific types. The types most frequently encountered were III, VI and XVIII.

The whole group of pneumococci was less prevalent in the late summer months than in the winter and early spring. No one type of pneumococcus showed any deviation from the general rule in seasonal distribution.

Poor economic conditions, with resultant overcrowding, did not, per se, increase the prevalence of any one specific type of pneumococci in contacts of pneumonia due to the homologous type.

The studies suggest that epidemics of family colds have some relationship to the prevalence of the homologous types of pneumococci in contacts of lobar pneumonia due to Type I and Type II.

LEUKOCYTES: An Unusual Hematologic Reaction to Neoarsphenamine, Rich, M. L. J. A. M. A. 101: 1223, 1933.

In a case of acute thrombocytopenic purpura following an injection of neoarsphenamine, a smear taken three hours after the injection showed, in addition to the thrombocytopenia, the presence of numerous degenerated neutrophilic cells. There was also evidence of intense marrow stimulation. These conditions quickly disappeared. It is concluded that neoarsphenamine at times has not only a toxic depressant action on the bone marrow but also a destructive action on some of the circulating elements of the blood, and that the neutrophilic cells as well as the platelets are susceptible to its action.

TUBERCLE BACILLI, Routine Culture of the Urine for, Seidman, L. R. J. Urol. 30: 2: 195, 1933.

This is a study of cultural methods in comparison with guinea-pig inoculation. The routine method used was as follows:

Ten cubic centimeters of the suspected urine, collected by catheter, are transferred by pipette, under sterile precautions, to each of 2 centrifuge tubes, and centrifugized at high speed for thirty-five to forty-five minutes. The clear supernatant fluid is then decanted, the sediment smeared on a clean new slide, and after fixation with heat, stained by Ziehl-

Neelsen. If no acid-fast bacilli are found after careful search a guinea pig is inoculated subcutaneously in the groin, with an emulsion of the remaining sediment in 1.0 c.c. of normal saline. The guinea pigs are examined weekly for the development of a gross lesion at the site of inoculation, and enlarged inguinal nodes. When these become definite, the animal is killed, an autopsy performed, and the lesions examined for acid-fast bacilli. If gross lesions do not develop, guinea pigs are killed, and autopsies done two months after inoculation.

All specimens, whether positive or negative, were cultured.

From a study of 89 specimens from 45 cases the following conclusions were drawn:

Eighty-nine specimens, from 45 cases, 81 of them bladder or kidney urine, were cultured. Tubercle bacilli were isolated from 60, or 67.4 per cent, of the specimens, and from 39, or 86.6 per cent of the cases. In 14 of the positive cultures, no acid-fast bacilli had been seen in the direct smear of the concentrated sediment. From 14 specimens, in which acid-fast bacilli had been seen in the direct smear, no growth was obtained. In 15 specimens, in which both culture and direct smear were negative, the inoculated guinea pigs developed tuberculosis.

Growth of tubercle bacilli was obtained on Corper's crystal violet potato cylinder from 54.0 per cent of the positive sediments, on Petroff's coagulated egg medium (without gentian violet), from 69.1 per cent, and on Sweany's milk meat infusion, egg and cream medium from 71.6 per cent. Corper's potato is a more satisfactory medium than these results indicate.

Five per cent oxalic acid, and 3 per cent sodium hydroxide proved better as digestants than 6 per cent sulphuric acid, both in the percentage of isolations, and in the average time required for the appearance of growth. All 3 reagents were equally efficient in destroying secondary contaminants.

The shortest interval for the primary isolation of tubercle bacilli by culture was sixteen days. Growth was obtained within three weeks in 18.3 per cent of the positive specimens, on 7.0 per cent of the inoculated culture tubes.

For the reasons discussed, guinea pig inoculation still appears to be superior to cultures for the diagnosis of tuberculosis in suspected cases.

No avian strains of tubercle bacilli were recovered. Strains from 18 cases were of the human type, and the remainder showed the growth characters of the mammalian organisms.

LUNG, Primary Carcinoma of, Hruby, A. J., and Sweany, H. C., Arch. Int. Med. 52: 497, 1933.

An epidemiologic and clinical study has been made of primary cancer of the lung, with reports of 13 cases, 12 of which were unquestionably primary in the lungs and bronchi and 1 primary in the ovary. There has been an approximately tenfold increase in the number of cases of cancer of the lung coming to autopsy within the last forty years, and a twofold increase in the last ten years. There is no valid evidence, however, to prove an increase in the general incidence of the disease except where there is contact with radioactive dust (Schneeberg and Joachimstal miners). The increase that has occurred may be partly accounted for on the basis of (1) the increased life expectancy from forty-three to fifty-eight years in approximately the last half century; (2) a better knowledge of the causes of other pulmonary diseases (e.g., the knowledge resulting from the discovery of the tubercle bacillus); (3) better diagnostic equipment (x-ray apparatus, bronchoscope and similar equipment); (4) increased zeal on the part of the medical profession and laity (better hospitalization, better transportation and other factors); (5) a changed attitude on the part of the pathologists in recognizing as primary carcinomas tumors that were once called metastases and sarcomas. It is not justifiable therefore to claim an increase in cancer of the lung until all the unknown factors are studied (such as the relative number of cancers of the lung that occur in rural districts).

For a diagnosis, reliance should be placed on a gradually appearing cough (commonly in men past middle life), followed by a variable but constant pain, and expectoration of sputum frequently streaked with blood, all of which may be accompanied with or followed by dyspnea.

Other less common signs are anorexia, fever, loss of weight, symptoms due to pressure (dysphagia and aphonia) and a variety of symptoms due to metastases. The physical signs reveal the presence of a gradually increasing bronchial tumor, with limitation of motion of the affected side, slight or no moisture at first, decreased breath sounds distal to the bronchial obstruction, hyperresonance above, dullness, and ultimately flatness over the tumor. The roentgenogram reveals a gradually progressing growth of tumor out from the hilus or along a bronchus, and the growth may be diffuse or circumscribed. The endoscopic view reveals a "woody" or fixed bronchus, and study of a bronchoscopic section generally clinches the diagnosis.

The laboratory examination should first reveal a scant or mucoid sputum, sometimes streaked with blood or tinted with hemoglobin, free from tubercle bacilli. Later, tumor cells should be found in the sputum or pleural fluid. Such early findings offer the only opportunity for permanent therapeutic aid.

A pertinent observation has been recorded pertaining to distant metastases from cancer of the lung. They seem to depend on the rapidity and extent of the growth of the primary tumor into a pulmonary vein.

UREA CLEARANCE, Test in Pregnancy, Cantarow, A., and Ricchiuti, G. Arch. Int. Med.
52: 637, 1933.

Determinations of urea clearance were made in thirty-nine cases of normal pregnancy and in seven cases of chronic glomerulonephritis and one of myocardial failure complicating pregnancy.

In the normal group, the urea clearance values ranged from 28 to 184 per cent of the average normal as established by Van Slyke and his associates.

The urea clearance, which was normal in the first few months of gestation, diminished as pregnancy progressed, being rather consistently low a few days before the onset of labor. High values were obtained during the early days of the puerperal period.

It is suggested that these findings may be dependent on changes in total metabolism and protein metabolism, fetal development and alterations in the rate and volume of blood flow through the kidneys.

It is further suggested that the laws governing the elimination of urea in the nonpregnant state, which form the basis of the mathematical calculation of the blood urea clearance, may not apply to the pregnant state because of the physiologic changes mentioned.

Subnormal clearance values obtained during the last two months of gestation must be interpreted with extreme caution, particularly in the absence of clinical or laboratory evidence of renal dysfunction.

CARCINOMA, Breast, The Bases for Histologic Grading of, Haagensen, C. D. Am. J. Cancer 19: 285, 1933.

In a series of 164 cases of carcinoma of the breast, which was exceptionally advantageous for the study of the relationship of histologic structure to prognosis because of the remarkably complete follow-up data and because of the superior quality and the large number of the histologic sections, a careful analysis has been made of the prognostic significance of fifteen different histologic characteristics. Six of these characteristics have been found to have a probable relationship to the end-result of the treatment. These significant characteristics are, in fact, similar to those which Hanseemann originally proposed for the determination of grade of anaplasia. The factors of fibrosis and lymphocytic infiltration, more recently stressed in tumor grading, have been found to be without prognostic import.

On the basis of these six significant histologic characteristics, it would appear that breast carcinomas can be classified into three grades of malignancy, in which the increasing grade of anaplasia parallels an increasing grade of malignancy as evidenced by the tendency of the tumor to metastasize and kill the patient at an early date.

It should be remembered that prognosis based on this type of histological evidence cannot claim a mathematical accuracy. It should be regarded only as an approximation, and a

rough one at that. The phenomenon of malignancy which we are attempting to measure is a biological one, and does not lend itself to exact measurement in mathematical terms. Moreover, the extremes, that is the Grades I and III, should be given more weight than Grade II, the less definite middle class into which a great proportion of breast carcinomas fall.

This knowledge gained from histologic grading should not be regarded as in any sense competing with the clinical data bearing on prognosis, to which it is, of course, subordinate in importance. Histologic grading should rather be considered as a new and additional form of information, as a modest but yet valuable increment to the knowledge of the disease.

The histological characters above referred to as having prognostic significance are:

1. Papillary character: origin in a cyst formed in a duct.
2. Comedo character: origin mainly within ducts, often with central necrosis.
3. Adenoid arrangement of cells: A, marked; B, slight; C, absent.
4. Variation in size and shape of nuclei: A, slight; B, moderate; C, marked.
5. Number of mitoses: A, few; B, moderate number; C, numerous.
6. Gelatinous degeneration.

DIABETES MELLITUS, Characteristics and Trends of Diabetes Mortality Throughout the World, Joslin, E. P., Dublin, L. I., and Marks, H. H. Am. J. M. Sc. 186: 754, 1933.

Analysis of available mortality data on diabetes in this country and abroad during the present century brings out these main features.

Diabetes as a cause of death has been rapidly growing in importance during the present century and is now one of the leading causes of death in this country.

The mortality from diabetes in this country is higher than in other parts of the world.

Diabetes death rates are increasing all over the world.

Most of the deaths from diabetes occur after middle life. Among children and young adults, the mortality from the disease is low. It begins to loom large about age forty and the rise in the death rates by age thereafter is rapid.

Up to age thirty-five present death rates of males and females are little different, but after that age the rates of women are the higher. This excess of the female death rate increases with advancing age up to sixty-five, when it is about twice that of males.

The high diabetes mortality in this country, as compared to other countries is not limited to one sex, but is found among both men and women. It is also found to exist at almost every age past thirty-five.

The diabetes mortality among negroes in this country has been increasing at a more rapid rate than among whites, and is now not much lower than that of whites.

SALMONELLA SUIPESTIFER, Infection in a Child, Haynes, E., and Meeks, L. T. Am. J. Dis. Child. 46: 1054, 1933.

It is apparent from a review of the cases described in the literature and the one reported here that infection with *S. suipestifer* does not produce any one characteristic clinical picture. Most commonly the gastrointestinal tract has been attacked. The symptoms and signs when this occurs sometimes resemble those of so-called food poisoning, such as is frequently caused by *S. enteritidis*; sometimes they are like typhoid or paratyphoid fever, and, in one case reported, the disease resembled bacillary dysentery. Next in frequency is involvement of the respiratory tract, where the infection may manifest itself as a bronchitis or as bronchopneumonia or lobar pneumonia. Symptoms referable to both the gastrointestinal and the respiratory tracts may occur together, as happened in the case reported. In addition to these more common manifestations, single examples have been reported of suppurative arthritis, sudden deafness, inflammation of the soft tissues around the jaw, puerperal infection with pleural effusion, peripheral neuritis and infarcts of the kidneys. Since infection with *S. suipestifer* can apparently cause a considerable variety of clinical manifestations, it seems advisable that in the investigation of any case of obscure fever, especially those associated with gastrointestinal symptoms, bacteriologic studies should be carried out with particular reference to this organism.

CALCIUM AND PHOSPHORUS, Blood, Concentration of in Serum of Normal Children,
Schoenthal, L., and Lurie, D. K. *Am. J. Dis. Child.* 46: 1038, 1933.

A study of the calcium and phosphorus of the blood serums of 250 normal children from five to sixteen years of age was made. There was no change in the level of calcium in relation to age. Phosphorus showed a significant decline, beginning with the fifteenth year of life. This occurred to a more marked degree in the girls than in the boys.

No clear-cut relation between the levels of calcium and phosphorus and growth was found.

The spontaneous variation of calcium and phosphorus was of the same magnitude as that seen after the ingestion of an ordinary breakfast.

Administration of viosterol in dosages of from 10 to 40 drops daily, or of 1 quart of milk, did not regularly increase the levels of calcium and phosphorus in these children.

PERITONEAL INJECTIONS, Changes in the Composition of Fluids Injected Into the Peritoneal Cavity, Schlechter, A. J., Cary, M. K., Carpentieri, A. L., Darrow, D. C. *Am. J. Dis. Child.* 46: 1015, 1933.

The changes occurring in electrolytes of various solutions when injected into the peritoneal cavity of dogs and guinea pigs were studied. The following solutions were used: (1) 0.9 per cent sodium chloride; (2) a mixture of sodium chloride, sodium carbonate and potassium chloride resembling interstitial fluid; (3) Hartmann's solution of sodium chloride, potassium chloride, calcium chloride and sodium lactate and (4) 5 per cent dextrose solution.

In each case the solutions injected into the peritoneal cavity underwent alterations in electrolyte concentration which indicate that they tend to approach the composition of interstitial fluid.

Electrolyte solutions were absorbed at an approximately constant rate, but the dextrose solution increased in volume before being absorbed.

It is pointed out that isotonic dextrose solutions undergo such great alterations in concentration and volume that subcutaneous or intraperitoneal injection probably is injurious to delicate tissues.

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The growth of human and bovine tubercle bacilli on good media is inhibited by normal blood from dogs or rabbits. This inhibition is more evident with small numbers of bacilli than it is with large numbers and it is more evident on inspissated egg yolk than it is on potato.

The effect is due to the development of toxic autolytic products from normal blood, and is absent when the blood has been treated with one or two volumes of 6 per cent sulphuric acid for one-half to one hour at 37° C., which destroys the autolytic enzymes, followed by neutralization with isotonic sodium bicarbonate solution (or other nontoxic alkalies). Regardless of the treatment, however, accurate determination of the number of tubercle bacilli placed in blood is not possible by counting the colonies which this blood will yield on good media suited to growing small numbers of human and bovine tubercle bacilli from fine suspensions.

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EDITORIAL

Sulphydryl and Cellular Multiplication

IN A SEARCH for further knowledge concerning the causation of cancer a group of investigators in Philadelphia have made a most interesting and important observation concerning the manner in which living cells are stimulated to reproduce themselves. The bearing of this observation on cancer is obvious if we realize that cancer is a disease in which the living cells of the body take on the capability of multiplying unrestrainedly and at the expense of surrounding tissues and organs. However, the immediate practical result of the research turns out to be in an entirely different field of medicine.

The investigation, conducted in the Research Institute of the Lankenau Hospital, was first directed to purely scientific attempts to study the division of living cells. The tissue that presented itself as being particularly appropriate for this was the root tip of the common onion. After a long period of investigation it became apparent that if some sulphur compound were made

readily available in the fluid in which the roots were growing the cells thereof would multiply more rapidly. Conversely in the absence of a sulphur compound reproduction was slowed down. It was found that the best sulphur compound for this purpose was the combination of sulphur and hydrogen, termed sulphydryl. Doctors Hammett and Reimann suspected that this might actually be the compound which stimulates cells to divide and multiply, since it is present in all living tissues.

While sulphydryl can in no way be looked upon as the cause of cancer, and as yet, no way seems clear to utilize this knowledge (such as by the restriction of sulphydryl in the food, or the administration of some substance that would neutralize its effect), for the practical application of this knowledge in the prevention or cure of cancer, the knowledge does represent one forward step in our halting progress of the study of this malady.

It is most interesting that glutathione, a substance containing a sulphydryl group in its chemical make-up, is present in all animal tissues. Glutathione contains the amino acids cysteine, glutamic acid, and glycine. The sulphydryl group occurs in cysteine. Cysteine is most abundant in the liver, an organ of unusual cellular activity and in epithelial structures, particularly hair. It is highly concentrated in wool and especially concentrated in human hair. It probably accounts in part at least for the rapid growth of human hair.

But it is equally interesting that it has been demonstrated that glutathione, with its constituent cysteine, is present in cancers in quantities almost as large as those found in the liver.

Hammett and Reimann have shown not only that the cells of the onion plant are stimulated by sulphydryl to reproduce, but likewise the normal tissue cells of man and the living cells of bacteria. Recently two independent workers at the National Institute of Health, Doctors Voegtlin and Chalkley, have demonstrated that glutathione serves equally well as a stimulant for reproduction of the ameba. The evidence appears to be accumulating that sulphydryl or some compound containing sulphydryl does actually stimulate multiplication of any type of living cell no matter what its nature.

The immediate practical application of this knowledge has been in the treatment of cutaneous ulcers which have either failed to heal or are healing very slowly under other methods of treatment.

Reimann applied dressings soaked with sulphydryl compounded with glucose (thioglucose) in the treatment of indolent ulcers which had resisted other methods. He found that while the edges of the ulcer tended to heal due to proliferation of the adjacent skin, bacteria infecting the wound also multiplied vigorously, apparently due to the sugar content of the thioglucose. He then changed his combination of sulphydryl, hitching it up chemically with an antiseptic, cresol, in the form of parathiocresol. He then achieved excellent healing. Later it was observed that glutathione itself would do as well.

Brunsting and Simonsen of the Mayo Clinic in an effort to find a substitute for the expensive glutathione discovered that cysteine itself was equally efficacious. They extracted cystine from human hair by hydrolysis and converted it into cysteine hydrochloride. This was used on the wet dressings ap-

plied to wounds. They found cysteine not only as efficient as thio cresol but less irritating to the adjacent skin and free from unpleasant penetrating odor of burnt rubber that is a feature of the latter.

The cysteine treatment of bed sores, ulcers following burns, slowly healing carbuncles, ulcers from injuries, ulcers from x-ray burns, serofulous sinuses, and the like is materially improved. Skin grafts show a decidedly better "take." In a series of 200 cases treated with cysteine, a decided stimulation to healing due to the cysteine was observed in 50 per cent, while in an additional 25 per cent, the beneficial effect was reported as promising. The cysteine treatment of ulcers is not a substitute but an adjunct to the other well-established procedures.

One can readily understand that sulphydryl compounds should not be used in the treatment of cancerous ulcers.

It is interesting in connection with the practical application of our knowledge of the action of the sulphydryl group in the treatment of the skin, to realize that the skin and its appendages are naturally rich in sulphur and also that dermatologists have long used sulphur, thus unconsciously attempting to stimulate the tissues of the skin to greater activity.

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—W. T. V.

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CLINICAL AND EXPERIMENTAL

EFFECTS OF LOW TEMPERATURE RETARDATION IN THE CULTURE OF STERILE MAGGOTS FOR SURGICAL USE*

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A LARGE number of laboratories, chiefly connected with hospitals, are now engaged in the culture of blowfly maggots for use in the Baer method¹ of treatment of infected wounds. In the cultural process it is necessary to hold back the rapid development of certain immature stages,² and it appears to be customary to do this by means of low temperature. Owing to the widespread use of this method of retardation and to observations made in the Bureau of Entomology which indicated that this procedure has certain limitations, an investigation has been made of the general effects of such retardation upon the normal functions and mortality of all stages held back in this way. A mechanical refrigerator running at from 40° to 43° F. was used. This range was found to be low enough to retard development, and is probably accessible to most laboratories. *Lucilia sericata*, the species commonly reared for the maggot treatment, was used in these experiments.

THE EGG

It is the usual practice to remove eggs from the fly cages in the afternoon and place them at once in cold storage for disinfection the next morning. It frequently happens also that a surplus of eggs is laid and they are stored until

*From the Division of Insects Affecting Man and Animals, United States Bureau of Entomology.

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needed. The length of time eggs may be retarded without injury, under various methods of storage, was tested in this series.

Source of Eggs.—All eggs used were produced under uniform conditions of temperature, moisture and food. Also, since the age of the female affects the viability of her eggs, the females were used only during the first three weeks of oviposition. Eggs were taken within two hours of deposition.

Methods of Storing.—(1) Eggs were left upon the meat and placed in covered Petri dishes. (2) Eggs were removed from the meat and placed in vials of water. (3) Eggs were removed from the meat and placed upon moistened cloth over a wet cotton pad in a covered Petri dish.

Since the eggs are usually laid in masses and are difficult to count, the weight method³ of determining number was used. Eggs were removed from storage at intervals up to 3 days and given a viability test.³

Effect of Age of Egg upon Survival.—It soon became evident that the degree of development of the embryo at the time of storage of the egg was important (Table I). When eggs freshly laid and up to two hours of age were

TABLE I
EFFECT OF AGE OF EGG UPON SURVIVAL IN COLD STORAGE

AGE WHEN STORED (HOURS)	NUMBER OF EGGS USED	NUMBER OF TESTS MADE	SURVIVAL (PER CENT)
0 - 2	3,623	6	27.6
1 - 3	2,134	3	73.1
2 - 4	3,138	6	82.1
3 - 5	2,009	3	82.5
4 - 6	3,910	5	74.8

(Normal* hatch, 85 per cent)

*The term "normal" used in each table refers to results when specimens are allowed to develop as checks in the ordinary fly cabinets without being stored.

placed in storage for twenty hours, only 32.5 per cent of the normal hatch occurred, whereas eggs two to four hours old when stored produced 97 per cent of the normal hatch. Therefore, only eggs which had been laid two to four hours were used. They were obtained by removing the meat from the fly cages after two hours and keeping it in the warm incubator for the additional two hours before storage.

Results.—Cold storage has a destructive effect upon the egg, as shown in Table II. Even the twenty-hour exposure, which is only slightly greater

TABLE II
LENGTH OF TIME EGGS CAN SURVIVE COLD STORAGE

TIME IN STORAGE (HOURS)	NUMBER OF TESTS MADE	NUMBER OF EGGS USED	SURVIVAL (PER CENT)
20	9	2,603	67.4
28	9	2,742	66.5
36	9	2,588	48.3
45	9	2,366	40.1
58	9	2,423	18.2
72	9	1,489	1.8

(Normal hatch, 77 per cent)

than the customary overnight storage, reduced the normal hatch by 12.5 per cent. The mortality was increased rapidly with length of exposure, and after three days' storage almost the entire number was destroyed. No difference in effect was observed among the three methods of storage. Mortality was greatly increased when eggs were removed directly from the fly cages to the refrigerator.

THE SURGICAL MAGGOT

Owing to the irregularity in the demand for sterile maggots a surplus frequently occurs. The expense of production would be reduced if any surplus could be stored safely until needed. Experiments were therefore conducted to determine the mortality under cold storage. The name "surgical maggots" is applied here to sterile maggots ready for use in wounds and about 4 to 6 mm. in size.

The maggots used in these tests were produced under the same sterile conditions as for use in wounds,² but in smaller lots of about 200 each. They were stored with some food in glass rearing bottles³ from one to six days.

Survival Tests.—As a check on their tolerance of cold storage, it was required that the maggots should be able not only to move when restored to the warmth of the laboratory but to resume feeding and growth. It is obvious that maggots which cannot feed are of no use clinically. Accordingly all maggots living when removed were placed upon raw beef to test feeding capacity.

Results.—A high rate of mortality occurs in cold storage of surgical maggots (Table III). The last column shows that even when stored only one

TABLE III
LENGTH OF TIME SURGICAL MAGGOTS CAN SURVIVE COLD STORAGE

TIME IN STORAGE (DAYS)	NUMBER OF EXPERIMENTS MADE	NUMBER OF MAGGOTS USED	NUMBER WHICH SURVIVED STORAGE	NUMBER ABLE TO RESUME FEEDING	SURVIVAL (PER CENT)
1	7	3,006	2,655	1,104	37.9
2	6	2,764	1,650	922	33.3
3	6	2,823	1,112	604	22.2
4	5	2,183	781	397	18.2
5	4	985	391	119	12.2
6	2	448	55	7	1.5
(Normal survival, 81 per cent)					

day less than 40 per cent were able to resume feeding. The mortality, increasing rapidly, amounted to almost 100 per cent in six days. Maggots reared under the same conditions but not stored had a normal mortality of only 19 per cent. Death of surgical maggots under the usual method of cold storage is therefore very considerable.

The fact that cold storage is very injurious to surgical maggots is evident not only in the number of maggots which were killed outright while in storage, but also in the inability of about one-half of the survivors to feed and continue their development. These latter losses easily escape detection unless closely observed. Cold storage of surgical maggots should therefore be avoided.

A Method of Avoiding Cold Storage of Surgical Maggots.—The only time that storage of maggots is essential in the cultural technic as ordinarily practiced is during the sterility tests,² when the growth of the maggots is held back in the refrigerator for forty-eight hours. Such retardation of growth by low temperature, however, is no longer necessary as a food has been devised by one of us (S. W. S.) which prevents the maggots from growing too large during the sterility tests. This food permits only a slow rate of growth throughout the cultural period, but does not interfere with normal feeding of the maggots when implanted in the wound. It consists of 1 part of evaporated milk to 5 or 7 parts of water, to which is added 1.5 per cent plain agar. This mixture is cooked in a double boiler for about twenty-five minutes and approximately 15 c.c. is used in each food bottle.

PREPUA

This stage is a convenient one for storing, as the larvae have already migrated from their food and are easily collected and handled.

Method of Storing.—Prepupae were taken from the sand about thirty-six hours after migration and while they still resembled larvae. The containers used were the common oiled paper or fiber cans, half-pint size, which are readily obtainable. They are waterproof and have a fairly tight-fitting lid. About one inch of dry sand was placed in each container along with the specimens. If a much larger quantity of sand is used, it should previously be chilled to storage temperature, otherwise the prepupae tend to develop to the pupal stage before the sand is cooled. It is important to prevent such development, as pupae are much less tolerant of storage than prepupae, as will be shown in the next section.

Loss of Weight During Storage.—Preliminary tests had shown that when no special precautions were taken to provide air-tightness in the containers, a noticeable loss of weight of prepupae occurred in storage. During the present experiments an investigation was made of the cause of shrinkage and methods of prevention. Specimens were stored in the containers under three different degrees of air-tightness. (1) Container was sealed with adhesive tape around the edge of the lid. (2) Container was not sealed and the lid fitted in the ordinary manner. (3) Container was the same as No. 2 but had numerous pinholes in the lid.

Each time specimens were removed from storage for survival tests, an equal number (twenty-four) were taken out and desiccated to constant weight at 100° C. for determination of weight losses.

Survival Tests.—Removals from storage were made at the intervals shown in Table V. The mere ability of prepupae to revive and move in the warm air of the laboratory was not considered sufficient indication of survival. Development into adults and oviposition of viable eggs by the females were considered necessary parts of the test. Accordingly, each lot when removed from storage was placed in a rearing cage, and the necessary care was given up to oviposition. When eggs were laid a viability test³ was made in each case.

Results.—The percentage of water and solid losses under the three methods

of storage are given in Table IV. Loss of water began at the outset of storage and continued throughout the period. Specimens stored in the sealed containers lost the least in both water and solids.

As to survival from storage, Table V shows that in two to four weeks no considerable mortality occurred, as 15 per cent is the normal death rate without storage. Holding beyond four weeks, however, caused a rather high death rate, and after nine weeks the mortality was 60 to 70 per cent.

The lowest mortality always occurred in the sealed containers. There is therefore a correlation between death rate and loss of weight in storage. Apparently when the water content is decreased below 33 per cent the mortality becomes especially high.

TABLE IV
EFFECT OF METHOD OF STORAGE UPON LOSS OF WEIGHT OF PREPUPAE

TIME IN STORAGE (DAYS)	PERFORATED CAP		ORDINARY CAP		SEALED CAP	
	WATER (PER CENT LOSS)	SOLID (PER CENT LOSS)	WATER (PER CENT LOSS)	SOLID (PER CENT LOSS)	WATER (PER CENT LOSS)	SOLID (PER CENT LOSS)
7	10.4	0.2	12.1	0.2	4.2	0
14	14.7	0.3	15.6	0.3	9.8	0
21	19.9	0.4	17.6	0.3	15.3	0
35	24.8	0.5	23.9	0.4	22.1	0.3
49	32.3	0.7	34.0	0.8	31.0	0.7
62	38.2	0.9	38.5	1.0	36.6	0.8
76	44.4	1.9	44.4	1.9	41.1	1.1
91	46.4	2.3	48.3	2.2	42.1	1.5
105	48.3	2.3	50.0	2.3	43.4	1.8

Another unfavorable aspect of cold storage of prepupae, which is not readily apparent, is the reduced egg-laying capacity of surviving females. In 9 colonies of flies which had been stored from three to four weeks as prepupae, it was found that the length of life of the flies was only one-half that of the preceding generations which had not been stored, and that the number of eggs laid was reduced to one-third of the normal.

TABLE V
EFFECT OF COLD STORAGE UPON PREPUPAE

TIME IN STORAGE (DAYS)	PER CENT SURVIVAL		
	PERFORATED CAP	ORDINARY CAP	SEALED CAP
16	83.5	67.8	83.5
33	60.5	54.2	66.6
49	45.8	45.8	62.5
62	29.1	29.1	37.5
76	18.3	24.1	37.5
91	18.3	19.1	20.8
105	12.5	8.3	12.5
(Normal survival, 85 per cent)			

THE PUPA

Pupae, being especially easy to handle and store, and requiring no food or attention, constitute a very convenient stage for storing. Reserve stock is frequently held in this stage.

Method of Storage.—The pupae were used when about thirty-six hours old. They were placed in the same three types of containers as used for prepupae. The same standard tests for survival were also made.

Results.—It soon became evident, however, that pupae could not be satisfactorily held over, as they were unable to endure even a moderate period of storage. After the second week the emergence fell to almost one-third of the normal, and most of the eggs which were laid failed to hatch (Table VI).

TABLE VI
SURVIVAL WHEN STORED AS PUPAE

TIME IN STORAGE (DAYS)	SURVIVAL (PER CENT)
7	66.6
15	37.5
22	15.2
29	11.1
36	8.2
43	2.7
(Normal survival, 86 per cent)	

After three weeks of storage the females which emerged died at an early age and laid no eggs. The type of container appeared to have no effect upon the results obtained with pupae.

SUMMARY AND CONCLUSIONS

In the production of surgical maggots it is customary to retard the rapid development of the immature stages by holding them in reserve under cold storage. An investigation has been made of the tolerance of the egg, surgical maggot, prepupa, and pupa to various periods of retardation.

Eggs may be kept in a closed container in a refrigerator at 40° to 43° F. overnight and up to twenty-eight hours with a mortality only slightly greater than normal, but continued storage is very unfavorable, and in three days results in almost complete destruction.

The age of the egg when stored affects the degree of injury. The lowest mortality occurred when eggs were allowed to remain in the warm incubator an additional two or three hours after removal from the fly cages before being stored.

Surgical maggots cannot satisfactorily be kept in cold storage. After twenty-four hours' retardation only 40 per cent were able to resume feeding, and in six days almost 100 per cent destruction occurred. A retarding food is described which eliminates the need of cold storage during the sterility tests.

Prepupae are best adapted to cold storage, but even in this stage the possibilities are considerably limited. They lose weight and become shrunken during cold storage. This is mostly water loss. Least unfavorable results were obtained with a relatively air-tight container. In thirty-three days the mortality was from 33 to 46 per cent and in seven weeks it had increased to approximately 38 to 55 per cent, depending upon the method of storage.

However, when prepupae were stored from three to four weeks the females which emerged were permanently injured and their egg-laying capacity was much reduced.

Pupae are unable to withstand even a moderate period of retardation. After the second week, mortality rose to about 66 per cent and most of the eggs subsequently laid failed to hatch.

Low temperature retardation of the various stages in the life cycle of the blowfly, although a convenience in cultural technic, causes such a high mortality that its use is considerably limited. It appears to be best adapted to short storage periods or when the convenience of continued storage is sufficient to counterbalance the losses.

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BACTERIUM MELANINOGENICUM²

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SINCE the brief description of *B. melaninogenicum* by Oliver and Wherry,¹ in 1921, there have appeared several studies of this organism. Attention has been focused on the subject because of two established facts, that the organism produces an abundance of a dense black melanin-like pigment, and that it is found in a variety of situations in the human body, both normal and pathologic. Pulmonary abscess is only one of the various pathologic conditions in which the organism is frequently encountered, but it is an example of a number of processes in which the etiology is obscure, and in which the developed affection represents more than likely a complex secondary infection. Since relatively little has been reported on *B. melaninogenicum*, since it normally exists in various parts of the body and thus is present when opportunity to invade exists, since it acts on blood substance, and since studies of pulmonary abscess here and elsewhere frequently revealed this organism in the abscess, the study of the type seemed to offer one of a number of possible avenues of approach.^{2, 3, 4, 5} The possibility of primary invasion was open. The potential rôle in infections in which the organism was a secondary

¹From the Department of Bacteriology and the Department of Surgery, University of California Medical School, San Francisco.

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Added by The Lillie Spreckels Wegforth Donation for the study of pulmonary abscess.

invader seemed worth study. The concept of the etiology and development of pulmonary abscess has been considered elsewhere.⁶

The purpose of the present report is to indicate the biologic characteristics of *B. melaninogenicum* determined in the course of the investigation above mentioned. It will be apparent that no evidence has been observed which would lead one to suppose that this organism is a significant etiologic agent in what becomes a mixed infection, nor that it has any significant part in the complex changes which obtain in the developed focus.

MORPHOLOGY

The organism is generally described as a small coccobacillus, gram-negative, nonsporulating, and nonmotile. In smears directly prepared from clinical material it frequently appeared as an encapsulated diplococcus (C. K.). In liquid medium pairs or clumps of the typical small cells appear, along with narrower rod forms and slender, slightly bent filaments, occasionally ten or more times the ordinary length. The organisms are somewhat larger and more definitely rodlike when grown on solid medium, and the morphology is more nearly constant. A considerable number of observations seem to indicate that the smaller rod forms take a bipolar stain or appear vacuolated. The cells are not acid-fast. Although measurements are likely to be misleading, those made on what was considered to be a normal or typical smear indicated a length of from 0.5 to 0.9 μ and a width of from 0.3 to 0.5 μ for the small types. Larger forms of 0.6 by 1.4 μ were not uncommon and these dimensions are not maximum.

Colonial morphology was not distinctive, except for pigment formation. Colonies appear smooth, shiny, moist, convex, and with an entire edge. Pigment varied from a light or deep coffee brown to jet black, and appeared microscopically to be accumulated densely at the center with a clear narrow surrounding zone. Moist colonies could be lifted off entirely, and older colonies were drier and disintegrated when touched with a loop. Hemolysis on blood agar was noted in pure cultures four to seven days old, at a point where growth was heavy or confluent. In mixed culture there appeared to be more marked hemolysis. Under good conditions, colonies attained a diameter of several millimeters.

ISOLATION

Clinical material from which this organism may be obtained frequently is usually heavily loaded with a variety of organisms. As elsewhere reported, although initial growth is not difficult, isolation in pure culture is hazardous. Heart hormone agar, P_H 7.4, with 5 per cent defibrinated rabbit blood, as suggested by Burdon,⁷ was satisfactory, as were other media. Freshly poured moist plates, inoculated immediately, were found to be most favorable. Slants, naturally moister, were used very successfully in rapid work. Plating technique, with repeated selection of colonies and transfer, was the only means found for isolation, although less time-consuming procedures were sought.

Other media on which growth was secured were:

1. Bordet-Gengou's medium.
2. Blood clot digest agar.
3. Veal infusion agar with 5 per cent rabbit or human blood; with human serum; with blood and 0.2 per cent cystine or 1 per cent glucose.
4. Brain broth with whole human blood; with human serum; with 30 per cent ascitic fluid.
5. Dehydrated (Difco) brain agar; with or without human whole blood or serum.
6. Extract ("Standard Methods") agar with rabbit or human blood, rabbit serum (P_H 6.4).
7. Liver hormone agar.
8. Semisolid (0.2 or 0.3 per cent) agar, with blood, serum, or ascites.
9. Loeffler's medium.
10. Beef heart broth with 0.05 or 0.1 c.c. whole blood per tube, or 30 per cent ascitic fluid; or without enrichment, with long established cultures.

No growth was secured with brain broth or veal broth or with dehydrated brain-heart broth (Difco) plus serum, blood, ascitic fluid, or adrenalin. Stimulating substances were not noted in experiments with autoclaved sputum, yeast filtrate, or carotin. In mixed cultures used in isolation a small amount of adrenalin^s seemed to produce larger and moister colonies, although no effect was noted with pure cultures.

Anaerobic conditions were necessary for isolation and growth, although the organism did not appear to be an absolutely strict anaerobe. The choice of method appears to depend on convenience. Hydrogen replacement (Boez), phosphorus jars (Varney), deep shake tubes, Veillon tubes, Fortner plates, Buchner tubes with pyrogallol, semisolid agar, deep brain broth tubes (enriched), Wright's tubes (and Petroff's plates prepared as Wright's tubes), and Krumwiede's plate method, all were successfully used.

SPECIMENS YIELDING B. MELANINOGENICUM

The organism has been described as common in the normal mouth and external genitalia, and in pathologic material from mixed infections. Isolations were made in the course of this study from the mouth, from a number of cases of pulmonary abscess, from seven uterine swabs, from one psoas abscess, from two ischiorectal abscesses, from one parotid abscess, from one submaxillary abscess, from one abscess following tooth extraction, from one open mastoid, from one colpotomy, from one liver abscess following perforation of gastric carcinoma, from one empyema, from one gunshot wound perforating the stomach, from one open leg ulcer, and from one perineum. Pigmented colonies were recognizable usually after four to twelve days, with an average of six days and a maximum of twenty-one days of incubation.

GENERAL PHYSIOLOGY

The optimum temperature for growth appeared to be 37° C. Exposure to 60° C. for ten minutes and longer rendered a seventy-two-hour broth culture nonviable. Heavy inoculations were found to be advisable for transfer, particularly in shifting from solid to liquid medium. The organism sometimes developed slowly on primary growth, and thereafter required frequent trans-

fer until well established, as Burdon suggests. Intervals of six to ten days were successful, and viability was demonstrated after twenty-four days' incubation in semisolid blood agar; after thirty-two to thirty-seven days in brain 30 per cent ascitic fluid broth, but not after sixty days in the refrigerator; and after twenty-eight days but not after six weeks when kept at 37° C. on solid medium. A very disagreeable odor of putrefaction was noted, somewhat cheesy with solid media, and butyric with liquid and semisolid amount of gas was observed in pure cultures in brain ascitic fluid broth and media (enriched with ascitic fluid), in which the odor was greatest. A small in beef heart blood broth one to three days after inoculation.

Fermentation, with acid and gas, was observed, after overcoming some technical difficulties, with dextrose. Sodium phosphate extract broth¹ enriched with ascitic fluid or rabbit serum, proved satisfactory. Not all media allow fermentation. Maltose, levulose, and mannite occasionally yielded acid without gas; sucrose, lactose, dextrin, and galactose were never observed to break down.

Using ZoBell's method⁹ no nitrate reduction was demonstrated; that is, nitrite tests were negative and nitrate tests remained positive. Indol tests were positive. Rather uniquely, brom cresol purple milk was acidified. It was also peptonized, as might be expected from proteolytic properties mentioned by Burdon. Slight liquefaction of Loeffler's medium was noted, partial digestion of brain particles in brain broth, and slight blackening of beef heart particles in beef heart broth after three or four days. Liquefaction of gelatin was not demonstrated although repeated tests were made, a discrepancy with reported results. Using brain 30 per cent ascitic broth, the liberation of H_2S was very marked.

PIGMENT PRODUCTION

The characteristic intense black pigment appeared to arise solely from blood substances. Repeatedly cultures produced pigment on blood medium, lost this property on medium without blood, although growing vigorously, and regained it when returned to a blood medium. In serum agar a late production of a reddish brown pigment appeared. Ascitic fluid and adrenalin appeared to be lacking in essential bases. Pigment appeared to be deposited after four or five days' growth (earliest noted, forty-two hours) and the development of a visible colony, first as a central brownish zone, later increasing to jet black and covering the whole colony. Bordet-Gengou's medium seemed especially favorable to pigment production, but no substance found chemically more definite than blood seemed to furnish the key to its development. When washed off with distilled water and dried on filter paper, it was found to be insoluble in distilled water, methyl alcohol, ether, chloroform, acetone, N/1 HCl, dilute ammonia; partially soluble in 10 per cent KOH (yellow solution, but pigment remained as granular masses), N/2 $NaCO_3$, and N/1 NaOH; soluble in acidified methyl alcohol (0.2 c.c. HCl to 5 c.c.) and in glacial acetic acid (pink color). Observations indicating that the pigment is toxic toward the organisms or that it is formed coincidentally with a toxic substance, were not convincing.

SEROLOGY

Extensive serologic tests were not considered advisable for the problem at hand. Agglutination tests, however, were performed with serum from rabbits given 11 injections intravenously in amounts of 1 to 4 c.c. of suspensions as high as 1 per cent. Antigen suspensions were made up to 0.25 per cent, preserved with Merthiolate. Agglutination set-ups were incubated two hours at 56° C., overnight at 37° C. and on ice for twenty-four hours. Control checks remained negative, and titers reached 1:1280 with +++ reactions, with some agglutination at 1:10,240 with one serum. Clumping was granular rather than flocculant. In support of this definite agglutination reaction a miscellaneous collection of strains was brought together and tested against specific anti-serum. Although the titers were not equally high with all strains, there was no reason to postulate the existence of a wide variety of serologically heterogeneous strains.

PATHOGENICITY

B. melaninogenicum was used, both in pure cultures and in mixed cultures, in experimental work on pulmonary abscess, the results of which will be reported elsewhere (Stevens and Shevky). No evidence was found that this organism had any invasive capacity, however, which is consistent with the observations mentioned herein. Burdon found⁷ with one strain an extensive cutaneous gangrene following subcutaneous inoculation, without septicemia, but with death in forty-eight hours. Most of the meager evidence available indicates a lack of pathogenicity.

Control checks were not extensive. Three each of rabbits, guinea pigs, rats, and mice were cultured around the gingival margins, and *B. melaninogenicum* was found in one rabbit. Bacteriologic studies of experimentally inoculated animals did not lead one to suspect that the organism was particularly common as a commensal in local stock animals.

Attempts to produce evidence of necrotizing properties by intracutaneous inoculation resulted in very minor or negative reactions. A suspension of organisms washed from a blood agar slant incubated fourteen days was matched with a 0.25 per cent typhoid bacillus antigen. The culture used originated from pulmonary abscess material seven weeks previously. Two series of three animals each of rabbits, guinea pigs, rats, and mice were used. Saline washings from blood agar slants were used as controls. The inoculum was 0.1 c.c. Consistent observation revealed several slight nodules or slight inflammation, but even these were insignificantly more marked than control reactions.

Attempts to induce invasion by intraperitoneal injection were performed with cultures from five to eight days old. Two series of 12 animals each, as in intracutaneous experiments, were used. Both blood agar and brain ascitic fluid broth cultures were used, with heavy inoculations (rabbits 5 and 10 c.c.; guinea pigs 3 and 5 c.c.; rats 3 c.c.; mice 1 c.c.) of standard suspension. Emulsions heated at 60° C. for two hours were used as controls. Observation was carried up to forty days. There appeared to be no evidence of

any kind, symptomatic, pathologic, or bacteriologic, of localization and multiplication of the organism. Since one series was carried through with densely pigmented growth and the other (broth culture) with no evidence of pigment, this property did not seem to be a factor in pathogenicity.

Intravenous inoculation of rabbits with large doses of both young and old cultures, variously made up, resulted in no abnormal temperatures or loss of weight, and the animals were continued with large repeated doses to develop an antiserum without apparent effect on their health. After final bleeding, autopsy revealed no significant changes, and cultures from the knee joint, the shoulder joint, right and left lobes of the lungs, liver, and spleen remained negative.

Attempts were made to introduce the organism directly into pulmonary tissue. Suspensions of young cultures were used, matched to both 1 and 2 per cent typhoid antigen. Quantities of 0.1 c.c. (rabbit), 0.08 c.c. (guinea pig), and 0.04 c.c. (mouse) were used in each series. The animals gained in weight and showed no abnormal temperatures during a month of observation. Autopsy revealed normal organs and negative cultures.

Intracerebral inoculations were given two series of animals, three guinea pigs and seven mice in the first, and four guinea pigs and two mice in the second. The animals were young, and several strains of young cultures were tried. Although maximum doses were used, there was no sign of any reaction whatever in any animal during a twenty-one-day period of observation. One mouse sacrificed in forty-eight hours yielded negative brain cultures.

SUMMARY

B. melaninogenicum appears to be common as a commensal and on surfaces normally or pathologically having a mixed flora. Detailed study suggests that this organism conforms to single type and is not one of a group of biochemically, morphologically, or serologically different types. All attempts, however, to show that it had any significance as a primary or secondary invader in mixed infections gave negative results, so far as clinical evidence or attempts in experimental animal infection could be correlated.

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THE TREATMENT OF RHEUMATIC CARDITIS WITH AQUEOUS EXTRACTS OF STREPTOCOCCI*

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RHEUMATIC fever has received a great deal of attention during the past decade but the problems of its specific etiology, prophylaxis, and treatment have scarcely advanced beyond the stage of hypothesis. This serves to establish special interest in the disease aside from that engendered by the vastness of its humanitarian, social, and industrial aspects. One theory regarding its specific etiology is, that streptococci are in some manner concerned as the causative agents. Concerning the manner in which they act, and the strain or strains which are responsible, there is a wide divergence of opinion.

This discussion of the treatment of rheumatic carditis will be limited to the use of aqueous extracts of streptococci administered by injection after the manner of vaccines. It will introduce a relatively new system of dosage of bacterial products which has been developed during the past six years by means of applying aqueous extracts of streptococci in the treatment of patients suffering from chronic atrophic arthritis.

The relationship between rheumatic fever and atrophic or rheumatoid arthritis is aside from this discussion. All that concerns it is, that both groups of patients present at least one feature in common. This is a marked reactivity, or hypersensitiveness to streptococci, or to their derivatives administered parenterally. This character has been designated "hyperergy" by Swift.¹ It is an outstanding feature of the "rheumatic state." Many types of streptococci have been used in demonstrating the existence of this hypersensitiveness, so that the type of streptococcus used in studying the reactions arising in patients appears to be less important than does the fact that it characterizes patients both with rheumatic fever and with rheumatoid arthritis.

The reactions excited in arthritis by the injection of extracts of streptococci² furnish an almost ideal means of studying the degree of this hypersensitiveness. An outstanding feature of the reactions in arthritis is an activation of lesions in the joints which are so superficially distributed as to permit direct observation of objective symptoms. Trial dosage if it produces a severe reaction does not endanger life even though it may increase considerably the patient's discomfort. On the other hand the patient with rheumatic fever presents reactions in the heart as well as in the joints, and for this reason cannot safely be treated with overdosage. After dosage and certain principles in the treatment of the hypersensitiveness of atrophic arthritis had been established, it was a relatively simple matter to apply them in the treatment of similar hypersensitiveness characterizing rheumatic carditis.

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DOSAGE OF THE EXTRACTS OF STREPTOCOCCI

The degree of this hypersensitiveness of the "rheumatic state" was determined by responses of patients with atropic arthritis to minute doses of aqueous extracts of streptococci. Throughout this study a saline extract of streptococcus cardioarthritidis was used under the designation of a soluble antigen.*

This product is prepared by suspending the streptococci in normal saline as in a vaccine. The concentration is relatively weak, being 100 millions per cubic centimeter. After seven days in a refrigerator, the bacteria are removed by filtration. The water clear menstruum containing the extractives is the undiluted soluble antigen. A series of sixteen dilutions of this are made, each successive one being one-tenth the strength of the last. The dilutions are conveniently designated as follows: 10^{-1} ; 10^{-2} ; 10^{-3} and so on up to 10^{-16} . A series of twelve dilutions only, beginning with 10^{-5} , and ending with 10^{-16} is used in treatment of arthritis, but only the last three, namely, 10^{-14} ; 10^{-15} ; and 10^{-16} are suggested in rheumatic carditis.

CHARACTERS OF THE REACTIONS

The reactions excited in this form of therapy are nothing more than aggravations of existing symptoms. They do not introduce new symptoms such as chills, high temperatures, etc., as are common in foreign protein therapy and which appear less frequently with the customary large doses of vaccines. They are triphasic but the triphasic character is brought out only by moderate overdosage. These three phases have been designated² "primary reaction," "period of euphoria" and "secondary reaction." They have also been observed by Crowe³ in his work with small doses of vaccine. He calls them "reaction," "response," and "relapse."

Proper dosage for the patient is followed by a period of euphoria beginning immediately upon the absorption of the antigen and lasting for a variable period of days (Chart 1). The outstanding features of this are relief of pain, buoyancy of spirits, and general stimulation. The euphoria terminates in the secondary reaction or "relapse," as Warren Crowe has more aptly named it. Administration of another suitable dose during the secondary reaction promptly terminates it by instituting another period of euphoria.

The primary reaction with moderate overdosage appears within the first twenty-four to forty-eight hours. It is usually followed by a period of euphoria which in turn terminates in the secondary reaction. It is a curious fact that further increases up to what may be termed gross overdosage do not proportionately increase the severity of the symptoms but rather tend to produce less stormy primary reactions which are delayed in their onset and prolonged in their course. These are referred to as "delayed primary reactions." They are not followed by periods of euphoria.

The delayed primary reaction begins forty-eight hours or more after an injection. During this interval no change is noted in the patient's clinical

*The soluble antigen used in this study has been supplied through the courtesy of Sharpe and Dohme, Philadelphia, Pa.

condition. It comes on gradually with symptoms which are of a general character rather than focal; e.g., malaise, lassitude, and generalized aching. It tends to be prolonged. Administration of another injection maintains the symptoms.

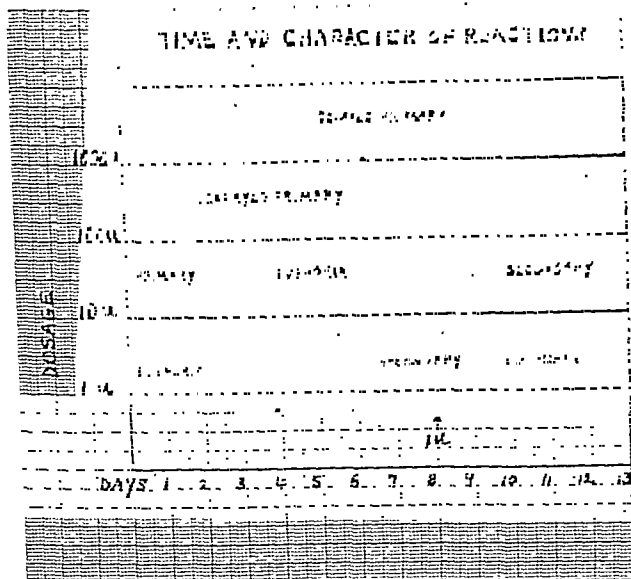


Chart 1.—In this diagrammatic chart the average level of the patient's symptoms is indicated by the solid lines at levels 1 u, 10 u, 100 u and 1000 u. The swing of the symptoms above or below this level is represented by the hatched lines. Suitable, or unit dosage of the extractives of streptococci is indicated at 1 u, and excessive dosages at 10 u, 100 u and 1000 u. The different reaction phases and their relations to the time of injection are illustrated.

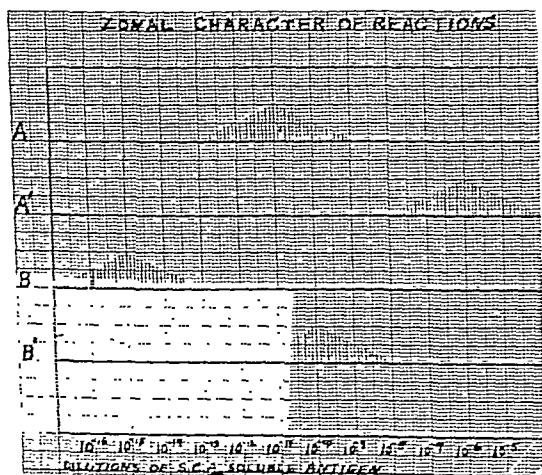


Chart 2.—At levels A and B are represented the zones of reaction of different patients following injections of the extractives of streptococci. At levels A' and B' the shifting of these zones to ranges of stronger dilutions is shown.

The secondary reaction follows a period of euphoria, usually of more than forty-eight hours' duration, during which there had been a well-marked improvement in the patient's clinical condition. It comes on abruptly with symptoms which are largely focal in type; e.g., increased pain, swelling, and red-

ness about the involved joints. It is terminated promptly by repeating the injection of the antigen.

ZONAL CHARACTER REACTIONS

The reactions are limited to a certain definite range of dosage. The administration of amounts below or above this range is not followed by reactions. This range varies for different individuals, and it shifts to stronger dilutions as treatment is continued in a given patient (Chart 2). This zonal character of the reactions is a very unusual phenomenon. Its recognition has great practical importance in adjusting dosage as will appear later under the discussion of the principles in the applications of treatment.

DISCUSSION

The extreme dilution of these extracts at first appears to be an absurdity. Experience over the period of years, during which repeated checks with blank solutions carrying only the saline and the traces of preservative used in extracts, have shown that contact with the streptococci imparts something not present in the blanks, which is potent in inducing reactions in arthritis even in these dilutions. The dilutions were arrived at gradually through trials of them in arthritis proceeding from the stronger ones down through the weaker ones. The guiding purpose was that of obtaining dilutions so weak that when convenient amounts were administered to patients with arthritis, no activation of the joints would follow. Encouragement was had from the fact that as the reactions were avoided, clinical improvement became more constant and more pronounced.

Another advantage of these minute dosages developed as the work progressed. They appear to act in the direction of desensitization only, perhaps because they are too small to increase the patient's reactivity. There is no need therefore to resort to intravenous injection as a precaution against this. This is a decided advantage over vaccines which even in small doses are prone when injected subcutaneously to reverse the process thereby increasing the patient's reactivity.

These doses are smaller than any yet used in biologic therapy. The nearest approach to them are those of tuberculin therapy. Others have used small doses of vaccines of streptococci in treatment of arthritis, but they can scarcely be compared with the ones used in this work. Warren Crowe,³ who has been using polyvalent vaccines of streptococci in arthritis for some twenty years, has arrived at a minimal dosage of 100 (only) microorganisms, and seldom goes above a dosage of 10 millions. Burbank⁴ states that some patients require initial doses of 50 (only) bacteria of polyvalent streptococcus vaccines. Each of these workers as experience was gained, has been reducing dosage.

PRINCIPLES IN THE APPLICATION OF THIS THERAPY

In contrast with treatment of foreign protein "shock" therapy, reactions must be avoided in this therapy if results are to be obtained. At all times to

avoid doses which are followed by reactions becomes the first principle of treatment. Since dosage may be above as well as below that range in which reactions occur, this principle must be further qualified by stating that dosage must be *below* that followed by reactions. This can be determined only by trial in the individual case, beginning with the weakest dilutions and gradually increasing dosage until a mild reaction appears. Thereafter dosage should be decreased slightly for best results. The continued use of doses within the zone of reactions aggravates the patient's symptoms. Persistent dosage above this zone, within certain limits, appears to influence the disease very little one way or the other.

The second principle is, that dosage to be most effective should be followed by periods of euphoria. It is not sufficient to avoid reactions but improvement must be obtained upon the absorption of the material injected. The optimum dosage is that amount which is followed by the longest period of euphoria. An amount which is followed by a period of euphoria lasting more than five days is regarded as satisfactory early in a course of treatment. If the euphoric periods are shorter than five days the dosage should be increased fractionally. The periods of euphoria tend to grow shorter under constant dosage during a course of treatment. When this occurs, it constitutes an indication for increasing dosage.

A third principle may be stated. Increase dosage only when the repetition of fixed amounts is followed by a shortening of the periods of euphoria. The periods of euphoria terminate in the secondary reaction. The appearance of this latter indicates that more antigen must be administered since the benefits derived from the last injection are at an end. Later in the course of treatment, as dosage is gradually built up the periods of euphoria lengthen, and secondary reactions grow milder. Finally the secondary reactions fail to appear as the arthritic process becomes quiescent.

The chief difficulty arising in practice is that of differentiating the delayed primary reaction of gross overdosage from the secondary or "relapse" reaction which calls for a repetition of the dose. One must rely on the symptoms presented in attempting to differentiate between the two reactions. If uncertain, the course of events following a repetition of the dosage usually suffices to clear up the doubt. Relief follows the repetition of dosage in the case of a secondary reaction and fails to do so in case of the delayed primary reaction.

PRESENTATION OF SELECTED CASES OF RHEUMATIC CARDITIS

With this information fairly well correlated and upon a background of experience in favorably influencing the "rheumatic state" as evidenced in arthritis, the problem of influencing in a similar manner the "rheumatic state" as seen in rheumatic fever has been approached by practically identical methods of practice. To present this work, a small group of 13 patients has been selected. These were chosen not because they demonstrate the most favorable results with this therapy but rather to illustrate certain errors which were made because of premature attempts to apply the method in rheumatic

conditions during the course of its development in arthritis. Thereby the parallelism between its applications in the two conditions can best be demonstrated. More recently all except one of these have received the benefits of dosage more properly adjusted to their needs. The observations on ten of them have extended over periods ranging from two to more than five years. The remaining three have been observed for less than a year, and serve to illustrate the results with the present methods. In three patients acute exacerbations followed injections of the larger doses which were employed from two and a half to four years ago. These followed an injection closely enough to suggest that they represented the primary type of reaction. Acute exacerbations have not occurred with dilutions of the soluble antigen weaker than 10^{-11} . They have also been absent as a spontaneous occurrence in patients treated by the weaker dilutions during the past two and a half years. In Table I are presented the summary of the outstanding clinical manifestations, the range of dosage used, and the results in each of the thirteen patients. Four (J. G., R. H., J. J., and T. K.) were seen first in acute attacks of rheumatic fever, or chorea, and treatment with the soluble antigen of streptococcus cardioarthritidis was begun with the passing of the acute symptoms. Six (E. H., K. N., S. W., W. R., A. L., and J. B.) were seen first during convalescence from a febrile attack of rheumatic fever or chorea. Three (R. McC., M. C., and M. L.) presented long-standing rheumatic disease of the heart, which was still active. Two of these had had no acute attacks of rheumatic fever or chorea. The other had two attacks of chorea and three of rheumatic fever. Only two patients were seen in their initial attack of rheumatic fever. The others, with the exceptions mentioned above, had had multiple attacks of rheumatic fever, or chorea. Eight of the patients were children with ages ranging from three years to thirteen. Three patients had active chorea when first seen, two had subcutaneous nodules, and another had a history of them with a previous attack of rheumatic fever. Three had pericarditis. One had acute iritis accompanying rheumatic fever with pancarditis. All had valvular cardiac lesions.

The range of dosage of the soluble antigen of streptococcus cardioarthritidis is charted opposite each patient's record in Table I. It will be seen that in the earlier applications of this antigen the doses were larger and that later ones were smaller. Three patients developed acute exacerbations and four others exhibited mild reactions to individual injections. All of these were among the earlier cases and all with the larger doses employed at the time. One of these patients died of cardiac failure in an acute exacerbation of pancarditis, the remaining six patients responded favorably when doses were reduced below the zone of reactions and have remained quiescent since.

The case (J. G.) is of interest because only mild reactions followed large individual doses, and the acute exacerbation occurred while dosage was being reduced. It represents an instance of tolerance to large doses and the entering of the zone of reaction from above. The other instances of acute exacerbations and of mild reactions to individual doses occurred while dosage was being increased. They are examples of entering the zone of reaction from below.

TABLE I. THE RANGE OF DOSAGES USED IN THE TREATMENT OF EACH PATIENT IS CHARTED

The range of dosages to which no reactions occurred is indicated by straight lines. The wavy lines show the range of dosages to which mild reactions occurred. Acute exacerbations are indicated by the hatched lines.

RHEUMATIC CARDITIS

Clinical Summary of Patients Treated.

	RANGE OF DOSAGE OF SCA SOLUBLE ANTIGEN										Age	Sex	Date	Diagnosis	Previous Attacks	Present Condition
	10^{-16}	10^{-15}	10^{-14}	10^{-13}	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}					
JG											8	M	6-21-27	R.F.; Pan. card. Subcut. nod.		Died 2/26/29
RH											21	M	5-24-27	R.F.; Aortic and Mit. regurg.	Two of R. F.	Quiesc. Aortic Mitral
EH											26	F	9-27-27	R.F.; Pan. card. Subcut. nod.	R. F. at 24 yrs. R.F.; Pericard. at 25	" Mitral
RMG											24	M	6-18-28	R. Card. Mitral and aortic	Chorea 6 & 10 yrs R.F. c 18, 11, & 24	" Mitral Aortic
JJ											11	F	6-22-30 8-1-32	Chorea; Mit. Sten.	Chorea 6 10 yrs. Per 21st, 10 & 11th yrs.	" Mitral
KN											11	F	8-11-30	R.F.; Pericard. and carditis	R.F. c 9 & 10 yrs.	" Mitral
SW											3	M	5-27-29	R.F.; Aortic & Mitral	None	" Mitral
TK											4	M	6-20-29	R.F.; Mit. regurg.	None	" Mitral
WR											11	M	6-24-30 11-6-31	R.F.; Mit. regurg. Mit. stenosis	R.F. 6 yrs. Recur. mild feb. attacks	" Mit. & Aort Cor. berrina
MC											36	F	11-1-30	R. card.; Aort. regurg. Mitral stenosis.	Tonill. & growing pains only	" Mitral & Aortic
AL											13	F	1-22-32	R. F.; Mitral regurg.	R.F. c 7 yrs; Tons. & grow. pains since	" Mitral
JB											12	F	3-28-32	Chorea; Aortic (?) & Mit. regurg.	Chorea 6 yrs; R.F. & subc. nod. 10 yrs	" Mitral
ML											43	F	9-8-32	R. card.; aortic Mit. stenosis	R. heart c 12 yrs Decomp. c 28 yrs	" Mitral

KEY OF SYMBOLS:—

— ZONE OF NO REACTIONS.

~~~~~ ZONE OF MILD REACTIONS.

||||| ACUTE EXACERBATIONS.

Patient J. J. is also worthy of special note since she was treated by repeated courses of injections of dilutions  $10^{-8}$  and  $10^{-9}$  during a period of two years without noteworthy effect on a persistent chorea of low grade. When dosage was dropped to the  $10^{-10}$  and  $10^{-15}$  range, the choreic symptoms disappeared and improvement in the general condition became evident after a few weeks.

In order to present more in detail the methods of this treatment Tables II and III are included.

TABLE II

K. N., AGED ELEVEN YEARS, AUG. 11, 1930. DIAGNOSIS: CONV. RHEUMATIC FEVER AND PERICARDITIS, RHEUMATIC CARDITIS, MITRAL REGURGITATION

| DATE     | WT.  | PULSE | HG | W.B.C. | SED.<br>R.B.C. | OP.<br>IND. | DOSAGE<br>S.C.A. ANT. | REMARKS                                                                           |
|----------|------|-------|----|--------|----------------|-------------|-----------------------|-----------------------------------------------------------------------------------|
| 8/11/30  | 57   | 120   | 70 | 10,700 | 36             | 0.9         | 0.1 $10^{-12}$        | Treated in office. Rest in bed all day                                            |
| 8/18/30  | 60   | 92    |    |        |                |             | 0.3                   |                                                                                   |
| 8/28/30  | 65½  | 90    |    |        |                |             | 0.1 $10^{-11}$        |                                                                                   |
| 9/ 4/30  | 67½  | 90    |    |        |                |             | 0.3                   | On 9/9/ relapse. Temp. 103°;                                                      |
| 9/12/30  |      | 105   |    |        |                |             | 0.1                   | pulse 102; dil. heart. 9/13/                                                      |
| 9/17/30  |      | 105   |    |        |                |             | 0.1                   | norm. temp.; much improved                                                        |
| 9/24/30  |      | 90    |    |        |                |             | 0.1                   | Confined to bed                                                                   |
| 10/ 2/30 |      | 90    |    |        |                | 0.8         | 0.1                   |                                                                                   |
| 10/ 9/30 | 69½  | 84    |    |        |                |             | 0.1                   | Patient returns to office                                                         |
| 10/16/30 | 70½  | 80    | 70 |        | 12             | 0.7         | 0.1 $10^{-12}$        |                                                                                   |
| 10/23/30 | 71½  | 84    |    |        |                |             | 0.1                   | Rest in bed greater part of day                                                   |
| 10/30/30 | 73   | 96    | 70 |        | 13             | 0.7         | 0.1 $10^{-14}$        |                                                                                   |
| 11/ 6/30 | 74½  | 80    |    |        |                |             | 0.1                   |                                                                                   |
| 11/13/30 | 73   | 96    | 70 |        | 14             | 0.8         | 0.05                  |                                                                                   |
| 11/20/30 | 72½  | 92    |    |        |                |             | 0.05                  | Out of bed. Restricted activities                                                 |
| 11/26/30 | 72½  | 74    | 80 |        | 27             | 1.0         | 0.05                  |                                                                                   |
| 12/ 4/30 | 73½  | 74    |    |        |                |             | 0.05                  |                                                                                   |
| 12/11/30 | 75½  | 78    |    |        | 18             | 0.7         | 0.05                  |                                                                                   |
| 12/18/30 | 76½  | 78    | 80 | 12,000 |                | 0.7         | 0.05                  |                                                                                   |
| 12/29/30 | 78   | 72    |    | 9,500  |                | 1.1         | 0.1                   |                                                                                   |
| 1/ 8/31  | 78½  | 81    |    |        |                | 0.5         | 0.1                   |                                                                                   |
| 1/15/31  | 78½  | 78    |    |        |                |             | 0.1                   |                                                                                   |
| 1/22/31  | 79½  | 84    |    |        |                |             | 0.1                   |                                                                                   |
| 1/29/31  | 79½  | 78    | 65 |        | 11             | 0.9         | 0.1                   | Returned to school second half year. Week interval and dosage continued unchanged |
| 4/11/31  | 80½  | 78    | 80 |        | 12             | 1.6         | 0.1                   | Week interval and dosage advanced to 0.15 c.c. $10^{-14}$                         |
| 5/16/31  | 81½  | 78    | 80 |        | 15             | 0.9         | 0.15                  | 14 day interval began dosage 0.15 c.c. $10^{-10}$                                 |
| 7/ 7/31  | 82½  | 78    |    | 7,300  |                | 0.8         | 0.2                   | 21 day interval dosage 2 to 2.5 c.c. $10^{-14}$                                   |
| 1/ 8/32  | 86½  | 72    |    |        | 8              | 1.3         | 0.3                   | 28 day interval dosage 0.3 c.c. $10^{-14}$                                        |
| 11/ 4/32 | 101½ | 72    |    |        | 12             | 1.5         | 0.4                   | Dosage increased                                                                  |
| 12/ 9/32 | 104  | 72    |    |        |                |             | 0.5                   | Dosage increased                                                                  |

In Table II are tabulated the dosage, intervals between treatments, certain clinical and laboratory data in the case of K. N. who showed a relapse under treatment when dosages were being increased rapidly. While a gain in body weight and a decrease in pulse rate occurred from the first, a definite tendency to quiescence of the rheumatic carditis did not appear until the

## SMALL: RHEUMATIC CARDITIS

dosage was dropped from  $10^{-12}$  dilution to  $10^{-14}$  dilution. Amounts of 0.05 c.c. to 0.1 c.c. of the latter were, in the light of further experience, still too large so that not until after treatment had been carried out for about five months was progress regarded as satisfactory. Apparently by this time a tolerance to a dosage of 0.1 c.c. of dilution  $10^{-14}$  had been established. This long pre-

TABLE III

W. R., AGED ELEVEN YEARS, NOV. 6, 1931. DIAGNOSIS: RHEUMATIC CARDITIS, AORTIC REGURGITATION, MITRAL REGURGITATION

| DATE     | WEIGHT | PULSE RATE | HG | SED. R.B.C. | DOSAGE 10-15 | REMARKS                                                                            |
|----------|--------|------------|----|-------------|--------------|------------------------------------------------------------------------------------|
| 11/ 7/31 | 84½    | 108        | 73 | 35 mm.      | 0.05         | Treatment in office. Activities greatly restricted. W.B.C. 9,800 Opsonic index 1.0 |
| 11/13/31 | 85     | 112        |    |             | 0.1          |                                                                                    |
| 11/20/31 | 84½    | 96         | 75 | 26 mm.      | 0.1          |                                                                                    |
| 11/27/31 | 88     | 96         |    |             | 0.1          |                                                                                    |
| 12/ 4/31 | 89½    | 100        |    |             | 0.15         |                                                                                    |
| 12/11/31 | 89     | 96         |    |             | 0.15         |                                                                                    |
| 12/18/31 | 87½    | 96         |    |             | 0.1          |                                                                                    |
| 12/28/31 | 89½    | 96         | 88 | 16 mm.      | 0.1          | Return to school where use of elevator was arranged                                |
| 1/ 9/32  | 92     | 96         |    |             | 0.1          |                                                                                    |
| 1/23/32  | 94     | 96         |    |             | 0.1          |                                                                                    |
| 2/ 6/32  | 93     | 88         |    |             | 0.1          |                                                                                    |
| 2/20/32  | 95½    | 90         |    |             | 0.1          |                                                                                    |
| 3/ 5/32  | 96½    | 96         |    | 10 mm.      | 0.1          |                                                                                    |
| 3/26/32  | 94½    | 105        |    |             | 0.12         |                                                                                    |
| 4/16/32  | 97½    | 92         |    |             | 0.15         |                                                                                    |
| 5/14/32  | 97½    | 84         | 98 |             | 0.2          |                                                                                    |
| 6/11/32  | 100½   | 90         |    |             | 0.15         | Severe cold                                                                        |
| 7/ 6/32  | 98½    | 84         |    | 18 mm.      | 0.15         |                                                                                    |
| 7/29/32  | 98½    | 84         |    |             | 0.1          | After one month at shore                                                           |
| 9/ 9/32  | 104½   | 96         |    | 7 mm.       | 0.15         |                                                                                    |
| 10/15/32 | 106½   | 90         |    |             | 0.2          |                                                                                    |
| 11/11/32 | 108½   | 84         |    |             | 0.2          | Grippe 12/24 W.B.C. 8,400                                                          |
| 12/ 9/32 | 110    | 84         | 88 | 8 mm.       | 0.2          | Opsonic index 1.0                                                                  |
| 1/ 6/33  | 108½   | 78         |    |             | 0.2          |                                                                                    |

liminary period is not observed in cases where treatment is begun with a much smaller dosage. This is illustrated in the record of patient W. R. as presented in Table III.

It is desired to call attention to the intervals between treatments as employed in these patients. Treatment is begun at seven-day intervals. After a course of seven or more injections, intervals of ten days are employed. Later fourteen, twenty-one, and twenty-eight days are allowed to elapse between treatments.

The treatments at monthly intervals are continued for at least a year after the carditis appears quiescent by all criteria used in estimating its activity. This is a precaution against relapse in a disease of the persistent character amply demonstrated for rheumatic carditis. When treatment is stopped, it is discontinued preferably during the summer months or at a season of lowest prevalence of the acute manifestations of rheumatic fever. In addition to the clinical observations used in estimating quiescence or ac-

tivity of rheumatic carditis, laboratory determinations of the hemoglobin, of erythrocytes and leucocytes, and of sedimentation distance of erythrocytes are used in following the course of the disease. Since the extract employed has been prepared from a single strain of streptococcus cardioarthritidis, the opsonic index readings of the patient's serum against this strain have been found to be most helpful in the matter of regulating dosage. Overdosage is regularly reflected in its depression of the opsonic index.

#### PRACTICAL APPLICATION IN RHEUMATIC CARDITIS

The treatment of rheumatic carditis with the aqueous extract of streptococcus cardioarthritidis as developed may be briefly outlined. An initial dosage of 0.05 c.c. of dilution  $10^{-16}$  of the soluble antigen is injected subcutaneously. The injections are repeated at intervals of from five to seven days, using 0.05 c.c. each for the first three injections. If tachycardia, cardiac arrhythmia or mild rheumatic pains develop within the first forty-eight hours the amount must not be increased until these symptoms fail to appear following an injection. Therefore limit any increase of dosage to 50 per cent, or less of the amount last administered. The most favorable results are obtained when the dosage is adjusted so as to avoid reactions. Improvement manifests itself in the erythrocyte count, leucocyte count, the hemoglobin, the sedimentation distance of erythrocytes, and the opsonic index of the patient's serum against streptococcus cardioarthritidis after from four to six treatments. No improvement usually indicates too large a dosage of the antigen.

Evidence of continued improvement after six or eight treatments at intervals of from five to seven days should be followed by treatments at intervals of from ten to fourteen days. Later the intervals may be lengthened to three or four weeks. Treatments at monthly intervals should be continued for at least a year after all signs of activity have disappeared. Dosage begun at 0.05 c.c. of dilution  $10^{-16}$  is usually not carried above 0.1 c.c. of dilution  $10^{-14}$ . Dilutions stronger than  $10^{-14}$  are not regarded as necessary and are not recommended in rheumatic carditis.

#### SUMMARY

There is a range of dosage of the aqueous extracts of streptococcus cardioarthritidis in which dangerous exacerbations of rheumatic fever occur.

In the patients observed to date such exacerbations have not occurred with a range of dilutions weaker than that designated dilution  $10^{-11}$  streptococcus cardioarthritidis soluble antigen, and mild reactions to individual doses occur infrequently in the range of dilutions weaker than dilution  $10^{-13}$ .

When dosages have been kept below those amounts followed by mild reactions to the individual injections clinical improvement has occurred regularly.

Dilutions  $10^{-16}$ ,  $10^{-15}$ , and  $10^{-14}$  only are recommended in the treatment of rheumatic carditis.

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## THE LIGHT FILTERING INDEX OF BLOOD SERUM\*

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### A DISCUSSION OF ITS CLINICAL APPLICATION

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IN A PREVIOUS paper,<sup>1</sup> it was suggested that the power of human blood serum to filter out light might possibly be utilized as a prognostic aid. The present paper records a technic which renders this suggestion practical, and presents a discussion of the procedure as applied to medical conditions.

Inasmuch as both the method of examination and the theoretical basis upon which the procedure under discussion is founded are somewhat novel, it seems advisable to consider certain aspects of the matter in detail. We have to deal with two factors: (a) serum and (b) light. Viewed from the purely physical standpoint, serum is a colloidal system. If one be permitted to revert to an elementary presentation, a colloidal system may be said to be composed of particles which may, or may not, vary in size, and which are separated from one another by varying amounts of a substance of different physical characteristics than the aforementioned particles, this separating substance being termed interface. Picturing such an arrangement one may conceive that a light wave passing through the serum encounters the colloidal particles, and that these either block, absorb, or reflect the light, thus acting as a filter. The amount of light which is held back would vary according to the conditions within the colloid system, and these conditions are dependent upon the size of the particles, the amount and nature of the interface, and the arrangement of particles and interface.

The current concept of light is that it is energy in the form of a wave and that the color of the light is related to, or dependent upon, the length of the wave. The unit by which the length of the light wave is measured is the Ångström's unit which is one ten-millionth of a millimeter.

\*From the Achells Laboratory, Lenox Hill Hospital.

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It has been found by actual observation (1) that light of different wave lengths, when passed through a layer of serum of definite thickness, the intensity of the light being relatively constant, is held back in varying degrees. It is upon this observation that the method of examination is based.

The Pulfrich photometer equipped with two filters having as their center of light filtration the following wave lengths (plus or minus 100 Å units) is employed, 7,200 Å and 4,300 Å. The mechanism and operation of this instrument has been described<sup>1</sup> previously. A standardized cell having a depth (thickness of serum layer) of 2.5 mm. is used and this cell is so constructed that the width of the serum layer is 3 mm. The portions of the cell which are not actually the walls of the cell cavity itself are painted black to cut out extraneous light. Blood for examination is collected in a Wright capsule after finger puncture. To give a sufficient amount of serum the body of the capsule should be 3.5 cm. in length and the bore approximately 8 mm. Blood can also be obtained by venepuncture, but this method is not necessary. Hemolyzed sera should be discarded. The time of taking the specimen is not material, if not too soon after eating, provided that in any given instance it is taken at the same time in all subsequent examinations; for example, if the first determination is made on a specimen collected before breakfast or two hours after breakfast, then subsequent specimens should be collected at the same relative time of day. After the serum is separated from the clot it may be examined at any time within twelve hours.

The standardized cells of the photometer are filled, one with distilled water, the other with the serum to be examined. In the manner described in the booklet of instructions which accompanies the instrument, the amount of light which is passed by the serum with each of the specified filters is then determined. The reading obtained with the 4,300 Å filter is subtracted from that obtained with the 7,200 Å filter. The result thus obtained indicates the amount of light, of the band situated between the central points of the two chosen filters, which has been blocked out. This result is then divided by the number of 100 Å units present in the band; i.e., 29.

*Example.*—The reading with the 7,200 filter indicates the passage of 95 per cent of the light, while with the 4,300 filter, 40 per cent of the light is passed. Between these two filters then, 55 per cent of the available light has been lost (95 minus 40). Since in the band between 7,200 and 4,300 there are 2,900 units, 55 is divided by 29 giving a quotient of 1.89. The filtration index in this instance is 1.89, but since in the computations recorded in this paper the second decimal place is either advanced one if above 5 or discarded if below 5, the corrected index is 1.9.

Expressed concretely, the light filtration index is obtained by subtracting the amount of light passed by the filter with the shorter wave length from that passed by the filter with the longer wave length, and dividing the result by the number of 100 Å units in the band lying between the center points of the two filters.

Isolated determinations of the index are valueless both from the diagnostic and prognostic standpoint, a question which has been considered in

the first report. It is only if the index is determined daily, or at possibly slightly less frequent intervals, that the collected data become of value. In the present paper we shall consider the application of the light filtration index in a group of medical conditions, surgical conditions being considered in another paper by Dujat and Kahan. Based upon 129 single observations made on normal individuals, the light filtration index normally varies between 1 and 3.1 with an average of 2.1. The distribution of the varying indices over the range, as expressed in percentages of the group is shown graphically in Chart 1.

A series of six normal individuals were examined every other day for a period of fifteen days. The data of these cases is given in Table I. In Case 1 the index was constant over the entire period. In Case 2 the index was constant over the entire period, except for the seventh day, the drop of 0.1 being

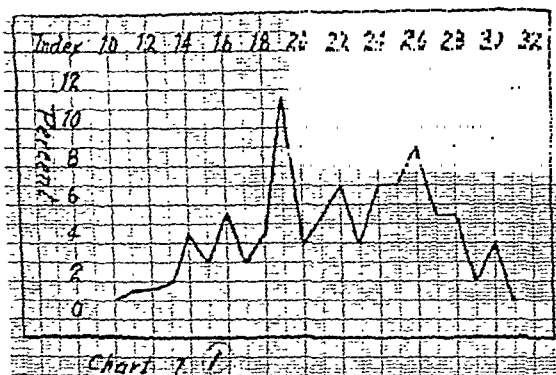


Chart 1

associated with constipation. In Case 3 the minor variations were associated with menstruation. In Cases 4, 5, and 6 the changes in the index were associated with constipation.

TABLE I

| DAYS   | 1   | 3   | 5   | 7   | 9   | 11  | 13  | 15  | MAXIMUM VARIATION |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-------------------|
| Case 1 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 2.2 | 0.0               |
| Case 2 | 1.9 | 1.9 | 1.9 | 1.8 | 1.9 | 1.9 | 1.9 | 1.9 | 0.1               |
| Case 3 | 2.7 | 2.7 | 2.5 | 2.5 | 2.7 | 2.8 | 2.7 | 2.7 | 0.3               |
| Case 4 | 2.0 | 2.0 | 2.0 | 2.0 | 1.9 | 2.0 | 2.0 | 2.0 | 0.1               |
| Case 5 | 1.9 | 1.9 | 1.9 | 1.8 | 1.9 | 1.9 | 1.7 | 1.8 | 0.2               |
| Case 6 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.4 | 0.1               |

*Examples of the Behavior of the Index in Disease.*—In a case of chronic nephritis (No. 3297, Chart 2) there was a marked nitrogen retention. On admission to the hospital the index was 1.4 at which time the patient was on a low nitrogen diet. He was then placed on a diet consisting solely of fruit juices, and within three days his index rose to 2.8 and promptly fell within twenty-four hours when he was again placed on a low nitrogen diet. During this period there had been no change in either the clinical condition or the blood chemistry. In the second, Case 9477 the clinical diagnosis was chronic

nephritis, hypertension and cardiac decompensation. As is shown in Chart 2 there were variations in the index with a gradual tendency over the period of seventeen days to reach a higher level. During this period, however, there was no decisive change either for better or worse in the patient's condition.

Changes in the index of a much more marked character were also observed in a case of Banti's disease (Chart 3, Case 35433) during an observation period of nineteen days. The significance of these variations could not be determined since the clinical condition of the patient remained practically unchanged.

In a case of hyperthyroidism (Chart 4, Case 35506) rather marked variations of the index were also observed during the fifteen-day period of observation. There was a general tendency of the index to run to successively higher

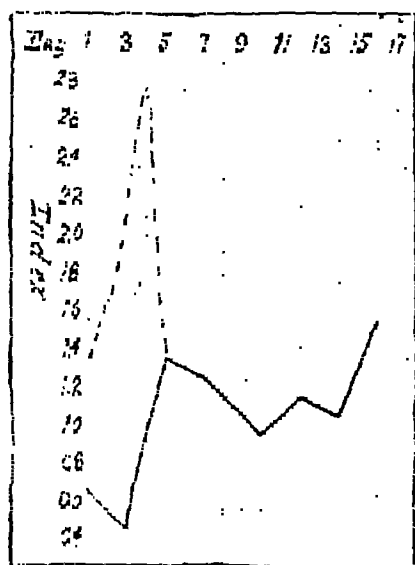


Chart 2. — 3477. — 2297

Chart 2

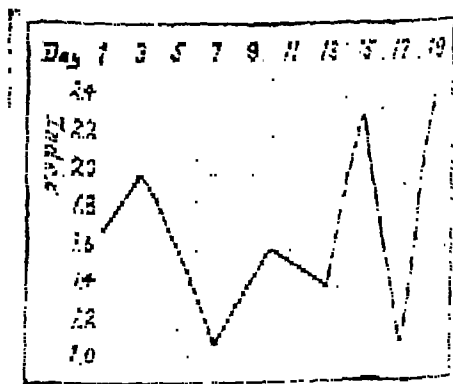


Chart 3. — 35433

Chart 3

values. This rise in the index was associated with a clinical improvement in the patient.

A further identification of the various factors which may influence the filtration index is afforded in Case 35043, Chart 5. This patient was admitted to the hospital with a diagnosis of chronic cholecystitis with cholelithiasis. The first index taken gave a value of 1.2. Five days later the index was again determined on a specimen taken about two hours after the administration of the dye used to demonstrate the gallbladder roentgenographically, and the index was found to be 3.0. Four days later the index had fallen to 1.5. Four days after the third index was determined the patient was operated upon and the index determined on a specimen taken twelve hours postoperation. The index had fallen to 0.6. The index was again determined three and seven days postoperation and a rise first to 1.8 and then to 2.7 was



demonstrated. The significance and application of the fall in the index directly postoperative and the subsequent rise is discussed in the subsequent paper by Drs. Dujat and Kahan.

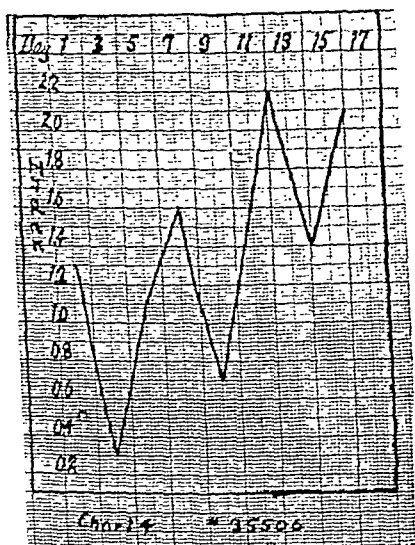


Chart 4

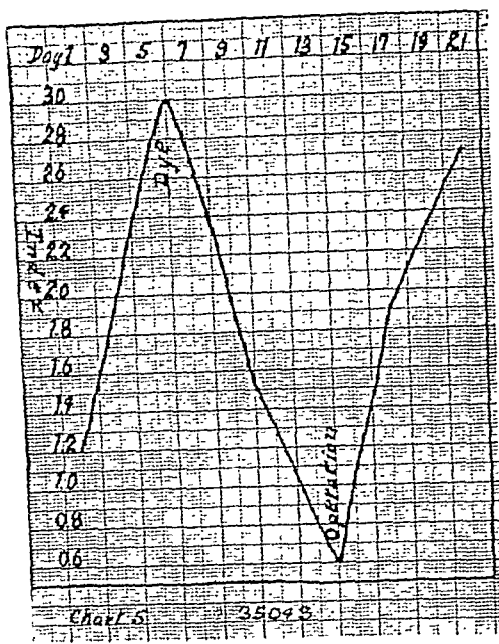


Chart 5

The influence of parenteral injection of foreign protein is shown in Case 35445, Chart 6. This patient was admitted to the hospital with a diagnosis of subacute arthritis of undetermined etiology. Vaccine therapy was given on the dates indicated. The termination of the vaccine therapy was followed by a sharp drop in the index.

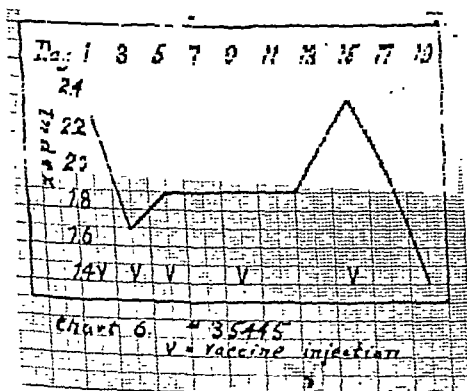


Chart 6

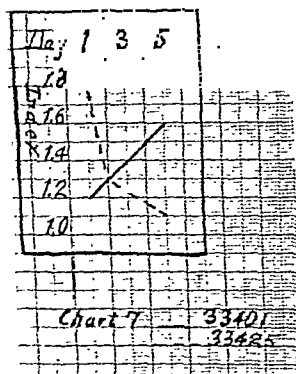


Chart 7

Suggestive of the use of the index in diagnostic differentiation is the curve in the Cases 33401 and 33425. Both of these patients were admitted to the hospital with a history of generalized pains, sore throat and fever.



Still another example is shown in Case 35531 (Chart 8). This patient with lobar pneumonia was admitted to the hospital on October 29. The first index was taken November 3 and was 1.3. The first day of completely normal temperature was November 5 when the index was 0.8. The indices on November 7, 8, 9, 10, 11, 12, 14, 15, 16, and 17 were as follows: 1.8, 1.3, 1.9, 1.1, 2.1, 1.9, 2.5, 2.5, and 2.5.

Still another example is given in Chart 9, Case 34501, one of pneumonia in which the index dropped from 1.3 to 0.7 on the first day of normal temperature. The index does not apparently change markedly previous to a decided change in the patient's condition which occurs suddenly as is shown in Case 33096, Chart 9. This patient had a lobar pneumonia and had an index of 2.0 just a few hours before a fatal cardiac collapse. Another case of lobar pneumonia, Case 35554, Chart 9, shows another phase of reaction of the index.

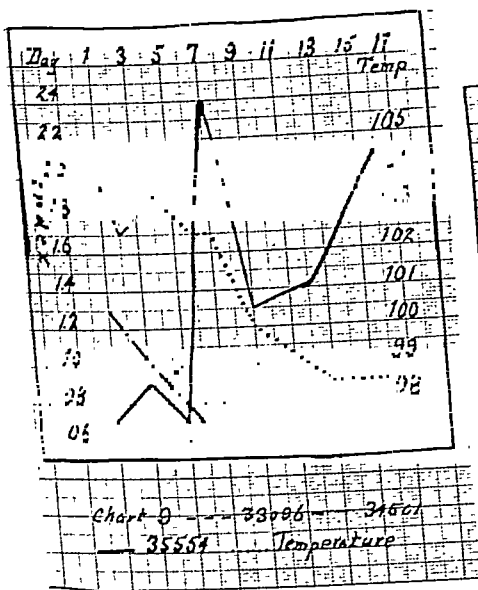


Chart 9

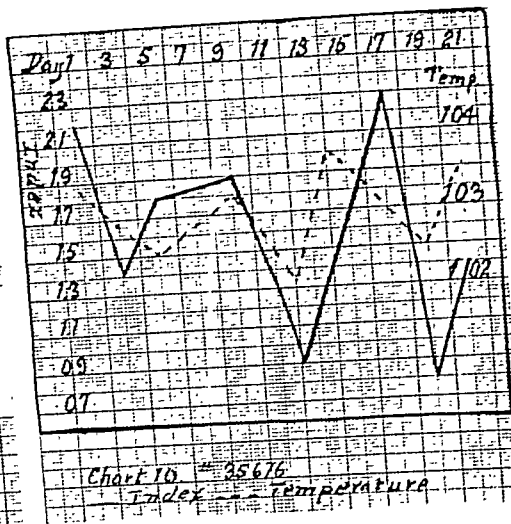


Chart 10

This patient who was very gravely ill had an index varying between 0.7 and 0.9, while the temperature was dropping from 105° to 102.5° F. With the temperature still at 102.5° F., there occurred a rather sudden and very marked improvement in the patient's clinical condition and coincidental with this the index rose to 2.4, to drop forty-eight hours later to 1.3. From this point the index rose steadily to 2.1, the rise antedating the return of the temperature to normal.

In long lasting bacterial infections, in which from the onset it is known that the outlook is unfavorable, as in bacterial endocarditis, fluctuations in the index associated with variations in temperature are also observed. Thus in the patient in Case 35676 (Chart 10) one of bacterial endocarditis, admitted to the hospital November 15 and dying February 4, this was observed. The first index was taken on December 1, and between then and December 21 the index was repeatedly determined. The relation of the temperature curve,

and the curve of the index is graphically shown in Chart 10. In the patient in Case 35428, also bacterial endocarditis, admitted to the hospital October 19 near the termination of the condition, the first index on October 26, was 0.8 while two days later it was 0.5. On October 30, a transfusion was given and while this has in most other instances been followed by a sharp rise in the index, it was in this case followed by a further drop to 0.4. Twenty-four hours after the time of the last index the patient died.

### CONCLUSION

If light of differing wave lengths be passed through nonhemolyzed blood serum a definite absorption of the available light occurs. The rate at which the light is absorbed, when calculated for bands 100 Å units in width has been termed the "light filtration index."

In normal individuals this index remains fairly constant over periods as long as fifteen days, minor variations occurring in consequence of simple physiologic disturbances such as constipation, menstruation, etc. The normal index varies between 1.0 and 3.1 with an average of 2.1.

The index is affected by the parenteral injection of foreign proteins, by the ingestion of colloids such as dyes and probably by still other factors.

In pathologic conditions associated with fever the termination of the fever is characterized by a sharp drop in the index and convalescence by a steadily rising index.

An index steadily between 2.2 and 2.6 is of good prognostic import as is a steadily rising index. A consistently low index is of bad prognostic import.

The changes which have been observed in the study of the phenomenon thus far warrant an intensive study in specific disease groups of the behavior of this biophysical change in blood serum so that definite laws guiding in the interpretation of the results may be established.

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# CHRONIC MYELOSIS IN CHILDREN\*

## WITH REPORT OF A CASE

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BAAR and Stransky<sup>1</sup> in their well-known *Klinische Haematologie des Kindesalters*, state that "myelogenous leukemia is well known, but exceedingly rare." In the age table of Reprint No. 143 of the Cancer Commission of Harvard University, the base of the age curve does not touch the 1-9 year line. For this reason we feel justified in reporting an additional case of this disease, and in presenting, in tabulated form, all the cases previously reported in the literature.

### CASE HISTORY

The patient was a Jewish boy, nine years of age, admitted to Sydenham Hospital on June 5, 1929, with six months' history of gradually increasing weakness, loss of weight and progressive increase in the size of his abdomen. Aside from slight shortness of breath, induced apparently by his enlarging abdomen, there were no other symptoms. At the beginning of his complaint he was seen by an outside physician who ordered a blood count, with the following findings: Hemoglobin 35 per cent, erythrocytes 2,140,000, leucocytes 425,000, thrombocytes 520,000. The differential smear showed 41 per cent immature neutrophiles, 4 per cent mature neutrophiles, 3 per cent eosinophiles, 3 per cent basophiles, 33 per cent neutrophilic myelocytes, 4 per cent basophilic myelocytes, 10 per cent myeloblasts, 1 per cent monocytes, and 1 per cent lymphocytes.

Six weeks before his admission to this hospital the boy was admitted to Mt. Sinai Hospital, where he stayed two weeks. An abstract received by us of his history there showed a diagnosis of chronic myelogenous leucemia.

The boy's past history showed a normal full-term delivery, weighing 11 pounds, with normal development thereafter. He was breast fed for one year and then put on the regular house diet. His childhood diseases included measles, chickenpox, and whooping cough. The family history was irrelevant.

Physical examination showed a young boy with good physical development and fair nourishment. He was rather pale, giving the appearance of one who is chronically ill. There was a slight dyspnea and orthopnea. He did not cough, and there was no cyanosis. His temperature by rectum was 101.6° F., pulse 116 per minute, and respiration 24 per minute.

The positive findings included marked deviation, to the right of the nasal septum. His eyeballs were large and protruded from their sockets. There were no other eye signs. There was marked pulsation of the vessels on both sides of the neck, and in the suprasternal notch, where a systolic thrill could be palpated. The thyroid gland was not enlarged. The heart appeared to be pushed up somewhat, the apex beat being in the fourth interspace inside the midclavicular line. There was diffuse precordial pulsation and a soft systolic murmur all over the precordium. The lungs were normal.

Examination of the abdomen showed it to be enormously distended, and the superficial veins were enlarged. On palpation an enormously hypertrophied spleen was felt reaching

\*From the Department of Pathology, Sydenham Hospital.

down to the symphysis pubis and extending over to the right side. To percussion there was only a small area of resonance, just below the liver.

The lymph nodes in the cervical, axillary, epitrochlear, and inguinal regions were all palpable as small granular masses.

Examination of the eyegrounds showed typical leucemic fundi with blurred, hyperemic discs, and highly dilated, tortuous veins. The arterioles were also dilated but to a lesser degree. There were no hemorrhages or exudative spots.

During the patient's stay at the hospital his temperature varied between 97 and 102° F.

Urinalysis at all times showed a trace of albumin with no other findings. The basal metabolic rate was +10. Blood chemistry was normal. A blood count at that time showed 28 per cent hemoglobin, 1,520,000 erythrocytes, 336,000 leucocytes, and 484,000 thrombocytes. The differential leucocyte count showed 10 per cent mature neutrophils, 16 per cent immature or band neutrophils, 20 per cent metamyelocytes, 12 per cent neutrophilic myelocytes, 20 per cent basophilic myelocytes, and 22 per cent eosinophilic myelocytes. Numerous blood



Fig. 1.—Authors' case. Autopsy. Note size of spleen. Weight 5,223 grams.

counts done at different periods during the next two years always showed a similar picture except at one time when the total leucocyte count was brought down to 50,000.

During that time the boy was admitted to the hospital several times and also watched through the dispensary, receiving several courses of benzol treatment and also of deep x-ray therapy to the spleen and long bones.

He received, in all, six transfusions, when warranted by his progressing anemia. They varied in amount from 300 to 500 c.c. and were all given by the Unger method of direct transfusion. The boy was of the Group 1 of the Jansky classification, or the class of universal donors. At each successive transfusion the reaction seemed to be more severe, and when on May 13, 1931, he was given 400 c.c. of carefully cross-matched blood from a professional donor, his temperature rose to 104° F. with a very rapid pulse, chill and hemoglobinuria. He died rather unexpectedly eight hours after the transfusion and approximately two and one-half years after his disease was first diagnosed.

Autopsy performed on April 14, 1931, revealed a large spleen and liver, the former measuring 37 by 21 by 10 cm. and weighing 5,223 gm., while the latter measured 27 by 23 by 8 cm. and weighed 2,680 gm. (Fig. 1). On section of the spleen the cut surface was angry-

red in appearance, with large outstanding granules. Microscopically, there was myeloid metaplasia and infiltration of the spleen, liver, kidneys, lungs, lymph nodes, and bone marrow (Figs. 2 and 3).

*General Features of Chronic Myelosis in Children.*—Mention has been made of the rarity of this condition. It is interesting to note that, rare as it is, it is exceedingly frequent when compared with chronic lymphadenosis in children. There is but one genuine recorded case of the latter condition. By far the

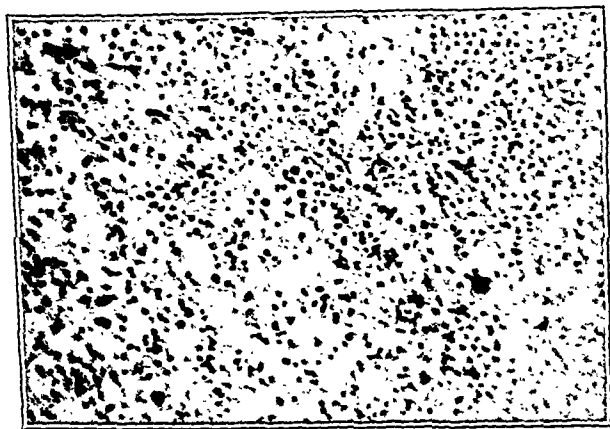


Fig. 2.—Spleen. Note absence of malpighian corpuscles and normal splenic architecture. Typical myeloid metaplasia present. (Authors' case.)

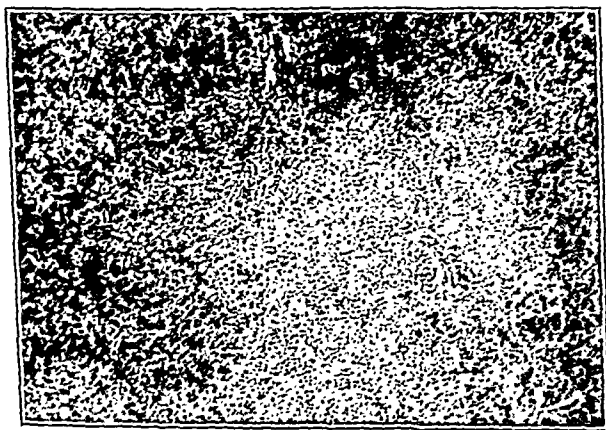


Fig. 3.—Liver. Note sinusoidal infiltration with myeloid cells. This choice of site for infiltration is typical of myelosis as contrasted with the periportal infiltration in lymphadenosis. (Authors' case.)

greatest incidence of leucemia in children lies in the acute types with the acute myelogenous leucemia even more prevalent than the acute lymphatic. The literature includes only 32 cases of chronic leucemia in children. The differentiation, however, between acute and chronic leucemias is often difficult.

It has been repeatedly stressed that in children, even more than in adults, the clinical difference between acute and chronic leucemia, lies not only in the duration of the disease but (and principally so) in the fact emphasized by

Fraenkel<sup>11</sup> that acute leucemias begin with those symptoms with which chronic leucemias end. It must be added, however, that in children chronic myelosis often runs a course so much more rapid than in adults, that on the duration alone one would be tempted to regard it as an acute case, were it not for the absence of the classical symptoms of acute leucemia, namely, hemorrhagic diathesis and an ulcerated mouth. One thing, however, is of great theoretical interest; when a case of chronic myelosis in a child develops the above-mentioned hemorrhagic and mouth symptoms, although the downward progress becomes much more rapid than in a true acute case, the blood picture remains typical of chronic myelosis, even though the case is now an acute one clinically.

Whatever our conception of acute leucemia and its relationship to the chronic form may be, chronic myelosis is a definitely independent clinicopathologic entity. It is a hyperplasia of the myeloid (granulocytic) tissue of the bone marrow and a metaplasia of the myeloid tissue in all the organs where embryonal totipotent mesenchyme is present, myeloid metaplasia in the spleen, liver, and lymph nodes. Hematologically, it expresses itself qualitatively in the presence of immature myeloid cells (granulocytes) from the myeloblast to the metamyelocyte, and quantitatively, in a very high leucocyte count, from 50 to several hundred thousand cells per cubic millimeter. The latter, the very high count, is not an essential feature, since the disease may run its entire course as a subleukemic myelosis with 20 to 50,000 cells, or even as an aleukemic form, with 1 to 10,000 cells per cubic millimeter of blood.

As to the etiology of this extremely interesting condition, though many attractive theories have been proffered, none have been associated with definitely proved facts, and we still know nothing that even begins to approximate certainty. The many hypotheses include the infection theory, as championed by von Hansemann, Manta, Mosler, Fontana and others, the belief that it is a neoplastic process, as claimed by Ribbert,<sup>26</sup> Banti, Warthin, Mallory and Piney, the tuberculosis theory, and lastly the theories of Ziegler<sup>21</sup> and of Naegeli<sup>23</sup> who believe that the disease is a disturbance of tissue correlation and can be traced to disturbed endocrinal relationships.

For a time the most alluring hypothesis was that which regarded all chronic leucemias, the myeloses and lymphadenoses, as a neoplastic process. Piney,<sup>26</sup> however, sounded the keynote of this theory when he said, "it seems the neoplastic hypothesis presents the *fewest difficulties*." True, from the viewpoint of monophasic anaplasia, leucosis would be regarded as a neoplastic process, but there are too many objections from other points of view, for such a theory to be more than a pretty parallelism. Some of these objections are the absence of infiltrative qualities in the invaded tissues, lack of close relationship to trauma or chronic irritation, the work of Ellerman in transmitting fowl leucoses, remissions and exacerbations, the nature of the hyperplasia, which involves the entire tissue system, over a wide area, rather than an organ, and finally the occurrence of the so-called "leucemoid" blood picture, at times indistinguishable from a true leucemia, yet returning sooner or later to the normal. One also recalls numerous authentic observations of leucemia where the



TABLE I

| AUTHOR                              | CASE | SEX | AGE | CLINICAL PICTURE                                                                                                                                                                                                                                      | BLOOD PICTURE                                                                                                                | NECROPSY |
|-------------------------------------|------|-----|-----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------|----------|
| Bass <sup>2</sup>                   | 1    | F   | 8   | One year history of loss of weight and appetite. Irritating cough. Pale, sallow, undernourished. Superficial nodes large, hard, discrete. Liver and spleen very large.                                                                                | Hg 41%<br>R.B.C. 2,080,000<br>W.B.C. 134,000<br>80% myeloblasts<br>5 neutrophils<br>15 lymphocytes                           | None     |
| Berghinz <sup>3</sup>               | 2    | F   | 8   | Nine months of small glands, spleen to pubis, large liver, some fever.                                                                                                                                                                                | R.B.C. 2,000,000<br>W.B.C. 800,000<br>27% myelocytes<br>59 neutrophils<br>2 eosinophiles<br>9 monocytes                      | None     |
| Cambell <sup>4</sup>                | 3    | M   | 6   | Three months of lymphomegaly. Liver large. Spleen enlarged to navel.                                                                                                                                                                                  | R.B.C. 2,200,000<br>W.B.C. 1,470,000<br>36% myelocytes<br>46 neutrophils<br>5 eosinophiles<br>10 basophiles<br>2 lymphocytes | None     |
| Cassell <sup>5</sup>                | 4    | F   | 8   | Palpable glands. Liver 2 cm. below costal margin. Spleen to umbilicus. Bones tender.                                                                                                                                                                  | R.B.C. 3,550,000<br>W.B.C. 500,000<br>69% myelocytes                                                                         | None     |
| Charon and Gratias <sup>6</sup>     | 5    | F   | 11  | Marked enlargement of liver and spleen.                                                                                                                                                                                                               | R.B.C. 880,000<br>W.B.C. 305,000<br>37% myelocytes                                                                           | None     |
| Duperie and Cadenaules <sup>7</sup> | 6    | M   | 9½  | Measles, pneumonia, whooping cough, bronchitis, enteritis bronchopneumonia prior to admission. Headaches, anorexia, abdominal pain, pallor, large liver, and spleen. Treated with x-ray and benzol. Died after four months with hemorrhagic symptoms. | Hg 50%<br>R.B.C. 2,410,000<br>W.B.C. 264,120<br>35% myelocytes                                                               | None     |
|                                     | 7    | F   | 13  | Began abruptly with pain in spleen. Loss of weight. Fever. In region of spleen 2 tumors both hard, large and immovable. Splenic puncture showed leucemic picture.                                                                                     | W.B.C. 206,410<br>reduced by deep therapy to 9,000<br>4% myelocytes<br>6 erythroblasts                                       | None     |
| Flesch <sup>9</sup>                 | 8    | M   | 13  | Seven months' history of fever with spleen enlarged to pubis.                                                                                                                                                                                         | R.B.C. 2,870,000<br>W.B.C. 230,000<br>26% myelocytes                                                                         | None     |
| Fowler <sup>10</sup>                | 9    | F   | 5   | Five and one-half months of moderate lymphomegaly with enlargement of liver and spleen.                                                                                                                                                               | R.B.C. 2,700,000<br>W.B.C. 240,000<br>13-30% myelocytes<br>39-69 lymphocytes                                                 | None     |
| Hutchison <sup>12</sup>             | 10   | F   | 5   | Five and one-half months of enlarging abdomen with enormous spleen. No liver and lymph node involvement.                                                                                                                                              | R.B.C. 2,425,000<br>W.B.C. 1,599,000<br>69% myelocytes                                                                       | None     |

TABLE I—CONT'D

| AUTHOR                             | CASE | SEX | AGE    | CLINICAL PICTURE                                                                                                                                                                                                | BLOOD PICTURE                                                                                                          | NECROPSY                                           |
|------------------------------------|------|-----|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|
| Japha <sup>14</sup>                | 11   | M   | 7½     | Sick for months. Began with swelling of abdomen and pain. Infantilism. Hard splenic tumor, smooth and tender. Leucemic retinitis.                                                                               | Hg 35%<br>R.B.C. 3,810,000<br>W.B.C. 261,000<br>Smear showed mostly granular cells with later many monocytes.          | None                                               |
| Koch <sup>15</sup>                 | 12   | M   | 10 wk. | Liver two fingerbreadths below the costal margin. Spleen reached to umbilicus.                                                                                                                                  | Hg 28%<br>R.B.C. 2,550,000<br>W.B.C. 20,900<br>Platelets 17,000<br>7% myelocytes<br>54 lymphocytes                     | None<br>This is a doubtful case.                   |
| Langsch <sup>16</sup>              | 13   | F   | 10     | Pain in left hip, left facial paralysis, ankle clonus, very large splenic tumor. Nerve and joint pains due to local leucemic infiltration. Much improvement under deep therapy.                                 | Hg 59%<br>R.B.C. 3,100,000<br>W.B.C. 224,000<br>48% myelocytes                                                         | None                                               |
|                                    | 14   | M   | 9      | Died with symptoms of hemorrhagic diathesis.                                                                                                                                                                    | W.B.C. more than 1,000,000                                                                                             | None                                               |
| Ledingham and Kerron <sup>17</sup> | 15   | M   | 11     | Four years of enormously enlarged spleen.                                                                                                                                                                       | R.B.C. 3,570,000<br>W.B.C. 234,000<br>48% myelocytes                                                                   | None                                               |
| Malmberg <sup>18</sup>             | 16   | F   | 8 mo.  | Father syphilitic. Liver large. Spleen to umbilicus. Septic fever. Hemorrhagic signs before death. Axillary node showed myeloid metaplasia. Duration ten months.                                                | Hg 28%<br>R.B.C. 1,550,000<br>W.B.C. 177,200                                                                           | None                                               |
|                                    | 17   | M   | 6 mo.  | Pallor at 5 months. Tenderness in head and body. Generalized lymphomegaly. Spleen to iliac crest. Liver enlarged. One and one-half years' duration.                                                             | Hg 35%<br>R.B.C. 1,800,000<br>W.B.C. 356,000<br>90% myelocytes and myeloblasts                                         | Typical chronic myelosis                           |
| Meyer and Heineke <sup>19</sup>    | 18   | M   | 4      | Insidious onset. "Gland" trouble at one year. Four weeks' enlargement of abdomen with occasional nasal hemorrhage. Diffuse adenomegaly. Numerous petechiae. Colossal spleen. Large liver. Death one year later. | Hg 52%<br>R.B.C. 3,080,000<br>W.B.C. 141,000<br>Many monocytes, eosinophiles, basophiles, and neutrophilic myelocytes. | Typical chronic myelosis. First case with autopsy. |
| Middleton <sup>20</sup>            | 19   | M   | 1½     | Eight months' history of weakness, loss of weight and abdominal swelling. Fever. Constipation and vomiting. Marked anemia. Spleen very large. No glands.                                                        | R.B.C. 3,330,000<br>W.B.C. 1 to every 9 R.B.C.                                                                         | Typical chronic myelosis                           |

TABLE I—CONT'D

| AUTHOR              | CASE | SEX | AGE | CLINICAL PICTURE                                                                                                                                                                                                                               | BLOOD PICTURE                                                                                                        | NECROPSY                       |
|---------------------|------|-----|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|--------------------------------|
|                     | 20   | M   | 4   | Six months' history of abdominal enlargement. No hemorrhages, vomiting or rickets. Spleen reached to umbilicus and to pubis. Heavy, hard with a smooth notch. No nodules.                                                                      | R.B.C. 3,000,000<br>W.B.C. 1 to ery<br>ery 5 R.B.C.                                                                  | Typical<br>chronic<br>myelosis |
| Morse <sup>21</sup> | 21   | M   | 1   | Gradual failure of health with enlargement of abdomen and inability to lie on left side. Marked anemia. Adenomegaly. Spleen filled entire abdomen.                                                                                             | R.B.C. 2,900,000<br>W.B.C. 48,000<br>22% myelocytes<br>Many erythroblasts                                            | Typical<br>chronic<br>myelosis |
| Morse <sup>22</sup> | 22   | M   | 4   | Six months of irregular fever with swelling of joints. Greenish pallor. Spleen to ant. sup. spine. Died suddenly one month after onset of enlarging abdomen.                                                                                   | No blood studies                                                                                                     | Typical<br>chronic<br>myelosis |
| Opitz <sup>24</sup> | 23   | M   | 7   | Sickly since age of one. Perirectal abscess, otitis media, gradual emaciation. Spleen and liver enlarged. Treated with sunbath and x-ray. At seven was undernourished, body was pigmented, spleen and liver large and hard. Died a year later. | At age of two, 80,000 W.B.C.<br>At age of seven, Hg 70%<br>R.B.C. 3,700,000<br>W.B.C. 46,000<br>51% myelocytic cells | None                           |
|                     | 24   | F   | 8   | At five noticed pallor, listlessness, loss of appetite, ascites and adenomegaly. Liver enlarged. Spleen 13 cm. in diameter.                                                                                                                    | Hg at one time was 2 per cent (sic!)<br>W.B.C. 37,000 to 460,000<br>Myeloblasts never over 5%                        | None                           |
|                     | 25   | F   | 10  | Facial paralysis. Left ankle clonus. Very large spleen. Ascites.                                                                                                                                                                               | W.B.C. 224,000<br>50% myelocytic cells                                                                               | None                           |
|                     | 26   | M   | 27  | Exudative skin manifestation. Indistinct speech followed by loss of speech. Large abdomen, cervical and inguinal adenomegaly. Liver to umbilicus. Spleen 2 cm. from midline and down to pelvis. Duration over six months.                      | W.B.C. 141,000<br>37% myelocytic cells<br>10% erythroblast                                                           | Typical<br>chronic<br>myelosis |
| Smith <sup>27</sup> | 27   | M   | 13  | Four years' duration. Spleen enlarged enormously, reaching into pelvis. Improved under benzol treatment.                                                                                                                                       | W.B.C. 372,000<br>Many myelocytes and eosinophiles. No myeloblasts. After benzol W.B.C. dropped to 10,000            | None                           |

TABLE I—CONT'D

| AUTHOR                                  | CASE | SEX | AGE | CLINICAL PICTURE                                                                                                                                                                                                                  | BLOOD PICTURE                                                                                         | NECROPSY                                                                              |
|-----------------------------------------|------|-----|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| Solnitz <sup>30</sup>                   | 28   | M   | 1   | Father a cured (?) syphilitic. Onset with increasing weakness and G-I disturbances. Spleen and liver large. No fever. Died one and one-half years later with hemorrhagic diathesis.                                               | Details not given except many myelocytes. Thrombocytes 35,000 with bleeding time of seventeen minutes | None<br>Malmberg <sup>15</sup> thinks this leucemoid reaction of congenital syphilis. |
| Steinbrinck and Stukowski <sup>31</sup> | 29   | M   | 12  | Began with sensation of fullness in stomach after eating. X-ray therapy brought quick and great improvement. Three years later recurred. Died one year later with symptoms of hemorrhagic diathesis.                              | R.B.C. 2,840,000<br>W.B.C. 304,000<br>26% myelocytes<br>64 neutrophiles                               | None                                                                                  |
| van Westrien-en <sup>33</sup>           | 30   | F   | 4   | Gradual onset with anemia, large spleen to pelvis, liver moderately large.                                                                                                                                                        | Hg 35%<br>R.B.C. 2,720,000<br>W.B.C. 528,000<br>54.1% myelocytes                                      | None                                                                                  |
| Authors' case                           | 31   | M   | 9   | Increasing weakness, loss of weight, increasing size of abdomen. Spleen filled entire abdomen. Improved under benzol, deep therapy, and transfusions. Course of two and one-half years with sudden death after sixth transfusion. | Hg 28%<br>R.B.C. 1,520,000<br>W.B.C. 336,000<br>Platelets 484,000<br>74% myelocytic cells             | Typical chronic myelosis                                                              |

blood picture would become normal under the influence of an intercurrent infection, and Jaffe<sup>13</sup> correctly stated that "these facts are difficult to reconcile with the conception that leucemia belongs to the malignant tumors." Finally, the tuberculosis theory is to be mentioned, only to be dismissed as being without any proof.

Despite the lack of positive evidence thereto, we still feel that the most possible progress toward discovering the etiology of this condition, will be made along the work of Ziegler and of Naegeli, on the basis that there is a disturbance in the endocrinal relationship with abnormality of the internal secreting organs.

## COMMENT

At the conclusion of the review of seemingly authentic cases, a few impressions appear justifiable. In the first place the mutual incidental relationship between chronic myelosis and lymphadenosis in children is the same, though markedly accentuated, as in adults, there being only one case of chronic lymphatic leucemia in children as against thirty-three of the myeloid type. Again, acute leucemias (which are much less common in adults than are the chronic forms) are, conversely, more frequent in children. So far as the aleucemic forms of leucemia are concerned, they are much more common, both in children and in adults, in myelosis.

In order of their frequency, therefore, the various forms of leucemia would appear as follows:

- CHILDREN
1. Acute myelosis
  2. Acute lymphadenosis
  3. Chronic myelosis
  4. Chronic lymphadenosis

- ADULTS
1. Chronic myelosis
  2. Chronic lymphadenosis
  3. Acute myelosis
  4. Acute lymphadenosis

In children, at least, the relationship of incidence according to sexes, is three males to two females.

In going over the clinical picture one becomes impressed with the fact that by far the most frequent complaint in early cases is "weakness, lassitude, listlessness and pallor." In more advanced cases it is "emaciation, enlarged

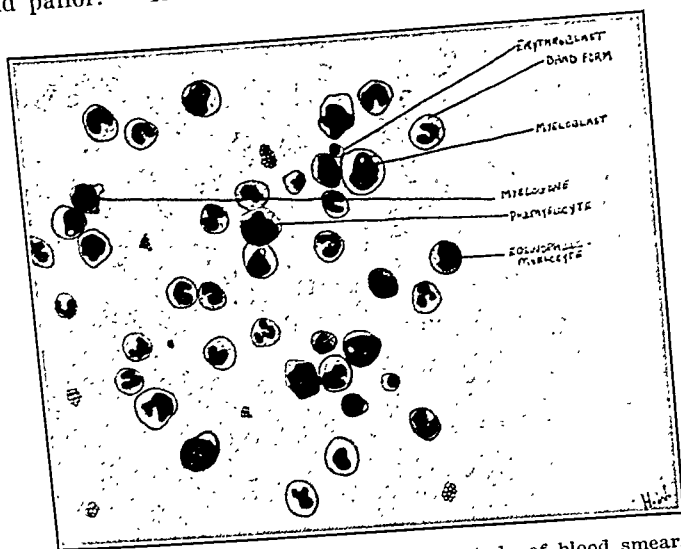


Fig. 4.—Authors' case. Drawing from oil immersion study of blood smear. Actual field.

abdomen" and very frequently "inability to lie on the left side." The spleen is always markedly enlarged, while hepatic enlargement varies from "slight" to "very marked."

A very striking feature of the history in a large majority of cases are numerous infections, from which the children had suffered previously, not infrequently with one infection following immediately upon another (see Cases 8 to 23). In fact a history of several infections succeeding each other at short intervals should always call for a thorough blood examination.

Adenopathy is not a frequent or a prominent symptom, and neither is fever.

The blood picture in chronic myelosis has been described innumerable times, and everyone is acquainted with the high leucocytosis, the immature granulocytes (metamyelocytes, myelocytes, promyelocytes and myeloblasts) and the increase in eosinophiles and basophiles (Fig. 4). In children, there is, perhaps, a greater frequency in the appearance of the youngest or myeloblast forms. This is probably to be accounted for by the great irritability of the hemo-

poietic system in children. The aleucemic form of the disease is quite rare. Anemia is always present and, frequently, is quite severe. It appears from the perusal of the literature that many cases reported as chronic myelosis were in reality cases of von Jaksch-Hayem disease.

Prognosis as to life is, of course, hopeless, and as to duration, variable, some cases lasting as long as five and six years, while others, especially those with a subleucemic count, lasted from a few months to two years.

In treatment, benzol, thorium and deep therapy are variable in their effects on different patients, but as a rule, they always react well to the x-ray treatment, the blood picture promptly changing toward the normal for varying periods of time. At times the anemia is so severe as to require several transfusions (authors' case). In connection with the latter it must be pointed out that toward the end of the disease accidents will occur despite the care with which the grouping and compatibility tests are carried out. Undoubtedly, they are due to some reaction on the part of the leucocytes, since according to Doan<sup>7</sup> there is a definite increase in the fragility of these cells in leucemias.

Just prior to death there almost always appear signs of hemorrhagic diathesis.

#### SUMMARY

1. There is presented an additional case of chronic myelosis in a child of nine years who responded to treatment for two years, and then succumbed following his sixth transfusion, due in all probability to the increased fragility of the leucocytes.

2. Thirty other authentic cases of chronic myelosis in children are collected from the literature and presented in Table I.

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## STUDIES ON THE QUANTITATIVE ESTIMATION OF BILE PIGMENTS\*

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IN 1916, Hooper and Whipple<sup>1</sup> developed a quantitative modification of Salkowski's<sup>2</sup> test for bile pigments whereby bilirubin and biliverdin were estimated together. They diluted specimens of bile with a nitro-hydrochloric acid alcohol mixture† and after about 18 hours estimated colorimetrically the characteristic blue green color that had developed on standing. In 1921, Rous and McMaster<sup>3</sup> introduced an inorganic standard‡ to replace the original artificial color wedge§, and later Sribhishaj, Hawkins, and Whipple<sup>4</sup> adopted the use of a monochromatic light filter in reading the intensity of the color in the solutions. Verifying the observations of Whipple and his associates, Riegel, Johnston, and Ravdin<sup>5</sup> in 1932 found that increasing the temperature hastened the reaction. They also found that the "characteristic blue green" color developed long before the maximum intensity was reached.

Further work in this laboratory on the estimation of bile pigment revealed significant inherent errors in current methods, the results of which emphasize the need for this study of the fundamental principles involved in the colorimetry of bile. For example, the time required to develop the color change with the acid alcohol mixture was far too variable to set any definite time at which to make colorimetric readings. One specimen of the acid alcohol may produce the complete color change in less than an hour while another may require more than a week. The color formed in from eighteen to

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‡Two c.c. conc. HCl, 0.1 c.c. conc. HNO<sub>3</sub>, and 100 c.c. 95 per cent ethyl alcohol.

§Ten c.c. 10 per cent copper sulphate plus 0.075 c.c. of 1 per cent potassium bichromate.

¶Made with copper sulphate, India ink, and gelatin agar. On cooling the wedge was sealed with vaseline and standardized against pure bilirubin.

twenty-four hours presents such a wide variety of green shades that frequently colorimetric comparisons are impossible without grossly violating the general laws of colorimetry.<sup>6</sup> At times, the color may be yellowish green, deep green, blue green, or blue. The standard of copper sulphate and potassium bichromate is a constant shade of blue green so that the shade of the unknown rarely matches that of the standard unless perchance by accident. There are also many different shades of blue green, and accurate comparisons are difficult, if not impossible, even when that color is produced. The original standard was prepared by Rous and McMaster<sup>3</sup> with 10 per cent copper sulphate to which they added, immediately preceding the quantitative colorimetric readings, more or less potassium bichromate as the occasion required in order to shift the tint of the standard to correspond with that of the individual unknown. It has been found that their procedure of changing the standard to match the shade of the unknown does materially alter the results. Sribhishaj, Hawkins, and Whipple<sup>1</sup> employing the Wratten Monochromatic Light Filter No. 72<sup>4</sup> found that readings could be made without delay, thus obviating the trouble of adjusting the standard to match each individual pigmentary solution. Their results involved the same error as Rous and McMaster's owing to the type of filter applied. The blue rays only are transmitted with Filter No. 72 which absorbs all other light rays, thereby obliterating the shade of color and permitting the observer to compare the blue intensity of any solution with that of a blue or blue green standard. Since the pigmentary value of the yellow intensity is not recorded, when varying quantities of yellow bichromate are added to the blue copper sulphate standard and when a light filter which records the blue intensity only is used, it seems reasonable to assume that the failure to record the yellow color is a source of an appreciable experimental error.

Although bile is potentially a polychromatic fluid, bilirubin is the parent chromogenic substance, and only those colors which are directly concerned with the oxidation reaction of pure bilirubin are considered in this study. When the pure pigment is dissolved in a simple oxidizing solution, the color change progresses from yellow through green and blue to an ultimately colorless solution. Proper classification of these colors is prerequisite to a better understanding of the colorimetric procedure which follows. The present scientific classification of all color is based on the discovery of the solar spectrum<sup>7</sup> in 1666 by Sir Isaac Newton, who found that sunlight was composed of many colors, which could be separated when the sun's rays were allowed to pass through a glass prism. Working with Newton's discovery, Sir David Brewster<sup>8</sup> in 1831 showed that the visible spectrum contained only three primary colors, namely, red, yellow, and blue. By means of light filters, which he had developed in 1822,<sup>9</sup> he demonstrated that the orange, green, and violet colors of the spectrum were secondary, produced by the overlapping of red and yellow, yellow and blue, and blue and red rays, respectively. The fundamental principle that green is a secondary color composed of two primary colors, yellow and blue, was utilized in the present study in the preparation of standards for the colorimetric estimation of all



green shades developed by the oxidation of bilirubin. This principle is applied by the quantitative separation of the yellow and blue intensities from a composite green solution by means of monochromatic light filters.<sup>2</sup>

*Experimental Procedure.*—A series of dichromatic green standards were prepared by combining definite quantities of yellow and blue dye solution.<sup>†</sup> The yellow dye was standardized against a known amount of pure unoxidized bilirubin<sup>‡</sup> and the blue against the maximum blue intensity developed from an equal quantity of oxidized bilirubin. These solutions were then mixed so that each successive standard contained the equivalent of a 10 per cent increase in blue and a compensating 10 per cent decrease in yellow. The resulting ratio of yellow to blue produced therefore a different shade of green in each member of the series. The Wratten Filter No. 76 was used for standardizing and reading the yellow in the solutions and No. 72 was used for the blue.

With these standards, an attempt was first made to ascertain the causes of the difficulties encountered with Hooper and Whipple's acid alcohol mixture, and second to devise an accurate method for determining bile pigment. A quantitative study of the color change in all phases of the reaction was developed along two major lines, namely, the factors which influence the reaction velocity and those which control the variation in shades. With the aid of light filters, experiments on the time required to complete the color changes were made with various oxidizing solutions as well as with different types of nitric acid as the oxidizing agent. Observations were also made upon the effect of temperature and light. The reaction velocity was determined by the amount of blue developed during any given period of time and the end

\*The Wratten Monochromatic Filter No. 72, when placed over the eyepiece of the colorimeter allows the observer to record only the intensity of the blue rays. With the Wratten Monochromatic Filter No. 76, one records only the intensity of the yellow rays.

These filters were obtained from the Eastman Kodak Co., Rochester, N. Y.

†Preparation of Standards.—

I. Stock solutions:

1. Pure bilirubin, 25 mg. in 100 c.c. of chloroform.
2. Dimethylamidoazobenzene, 32 mg. in 100 c.c. ethyl alcohol.
3. Nile blue sulphate, 7.5 mg. in 100 c.c. ethyl alcohol.
4. Nitrohydrochloric acid alcohol<sup>1</sup> made with strictly fresh nitric acid.

II. Standardization of diluted stock solutions:

1. Dilute 20 c.c. of the stock yellow dye to 500 c.c. with alcohol.
2. Dilute 20 c.c. of the stock blue dye to 500 c.c. with alcohol.
3. Expose the nitrohydrochloric acid alcohol solution to ultraviolet light for three minutes at 18 inches from the quartz mercury vapor tube. Stir the solution every fifteen seconds. The depth of the solution not to exceed 5 cm.
4. From a microburet, measure 1 c.c. of the stock bilirubin solution into each of ten 25 c.c. volumetric flasks.
5. Dilute 5 flasks to the mark, with plain ethyl alcohol.
6. Dilute the other 5 flasks to the mark with the treated nitrohydrochloric acid alcohol solution. Allow to stand one hour, at which time they should all be steel blue in color.
7. With the first 5 flasks of unoxidized bilirubin solutions as standards, determine the bilirubin equivalent of the yellow dye. If the average for the series does not closely approximate the known amount of bilirubin, adjust the intensity and restandardize. Use light filter No. 76.
8. With the second 5 flasks of oxidized bilirubin, determine the bilirubin equivalent of the blue dye. Restandardize if necessary. Use light filter No. 72.
9. From a buret measure accurately and mix the yellow and blue dye solutions as follows:

| Standard No. | 0   | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10  |
|--------------|-----|----|----|----|----|----|----|----|----|----|-----|
| C.C. Yellow  | 100 | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 | 0   |
| C.C. Blue    | 0   | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |

10. Label each standard according to its value in terms of oxidized and unoxidized bilirubin.  
<sup>†</sup>Obtained from the Eastman Kodak Company, Rochester, N. Y.

TABLE I  
A COMPARATIVE STUDY OF THE QUANTITATIVE ESTIMATION OF BILIRUBIN AS INFLUENCED BY  $\text{HNO}_3$

| Method 1: Estimations made with single copper sulphate and potassium bichromate standard with the aid of filter No. 72. Solution contains 1.040 mg. per cent bilirubin. |                           |             |               |               |                 |                                    |                |                 |                                            | Method 2: Estimations made with a series of dichromatic green standards with the primary colors mixed in an inverse ratio as described in the text. |               |                        |                 |                    |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|-------------|---------------|---------------|-----------------|------------------------------------|----------------|-----------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------|---------------|------------------------|-----------------|--------------------|
| SOLUTION NUMBER                                                                                                                                                         | OXIDIZING SOLUTION*       | TEMPERATURE | LIGHT         | REACTION TIME | COLOR           | ESTIMATIONS IN MILLIGRAMS PER CENT | PER CENT ERROR | STANDARD NUMBER | YELLOW INTENSITY (BILIRUBIN) FILTER NO. 76 | BLUE INTENSITY (BILIRUBIN) FILTER NO. 72                                                                                                            | BILIRUBIN PLS | PER CENT OF OXIDATION† | PER CENT ERROR‡ | STAGE OF OXIDATION |
| 1                                                                                                                                                                       | HNO <sub>3</sub> 1 yr old | Room        | Lab. Daylight | 15 min.       | Blue            | 1.099                              | + 5.67         | 9               | 0.068                                      | 0.990                                                                                                                                               | 1.058         | 95.1                   | +1.75           | Late 2nd           |
| 2                                                                                                                                                                       | HNO <sub>3</sub> Fresh    | Room        | Lab. Daylight | 24 hr.        | Green           | 0.320                              | -69.23         | 3               | 0.692                                      | 0.341                                                                                                                                               | 1.033         | 32.7                   | -0.67           | Middle 1st         |
| 3                                                                                                                                                                       | HNO <sub>3</sub> Fresh    | Room        | Dark Daylight | 24 hr.        | Yellowish Green | 0.321                              | -69.13         | 3               | 0.682                                      | 0.343                                                                                                                                               | 1.025         | 32.9                   | -1.43           | Middle 1st         |
| 3                                                                                                                                                                       | HNO <sub>3</sub> Fresh    | Room        | Dark Room     | 48 hr.        | Green           | 0.404                              | -61.15         | 4               | 0.585                                      | 0.452                                                                                                                                               | 1.037         | 43.4                   | -0.28           | Late 1st           |
| 3                                                                                                                                                                       | HNO <sub>3</sub> Fresh    | Room        | Dark Room     | 72 hr.        | Green           | 0.667                              | -35.86         | 5               | 0.533                                      | 0.490                                                                                                                                               | 1.023         | 47.1                   | -1.63           | Early 2nd          |
| 3                                                                                                                                                                       | HNO <sub>3</sub> Fresh    | Room        | Dark Room     | 7 days        | Blue            | 0.715                              | -31.25         | 7               | 0.276                                      | 0.753                                                                                                                                               | 1.020         | 72.4                   | -1.92           | Middle 2nd         |
| 4                                                                                                                                                                       | HNO <sub>3</sub> Fresh    | Room        | Lab. Daylight | 7 days        | Green           | 0.811                              | -22.01         | 8               | 0.225                                      | 0.827                                                                                                                                               | 1.052         | 79.5                   | +1.09           | Middle 2nd         |
| 5                                                                                                                                                                       | Same lot as No. 3         | 38° C.      | Dark Room     | 24 hr.        | Green           | 0.622                              | -40.19         | 6               | 0.379                                      | 0.672                                                                                                                                               | 1.051         | 64.6                   | +1.05           | Early 2nd          |
| 5                                                                                                                                                                       | Same Lot as No. 3         | 38° C.      | Dark Room     | 48 hr.        | Blue Green      | 0.901                              | -13.36         | 8               | 0.170                                      | 0.883                                                                                                                                               | 1.053         | 84.8                   | +1.25           | Middle 2nd         |

\*95 per cent ethyl alcohol

\*95 per cent ethyl alcohol 100 c.c.; HCL 2 c.c.;  $\text{HNO}_3$  0.4 c.c.

†Calculated from blue intensity.

‡Calculated from both yellow and blue.

TABLE I—Continued

| SOLUTION NUMBER | OXIDIZING SOLUTION*                       | TEMPERATURE | LIGHT         | REACTION TIME | COLOR | ESTIMATIONS PER CENT |                     | STANDARD NUMBER | YELLOW INTENSITY<br>(BILIRUBIN)<br>FILTER NO. 76 | BLUE INTENSITY<br>(BILICANIN)<br>FILTER NO. 72 | BILIRUBIN PLUS<br>BILICANIN | PER CENT OF<br>OXIDATION† | PER CENT ERROR† | STAGE OF<br>OXIDATION |
|-----------------|-------------------------------------------|-------------|---------------|---------------|-------|----------------------|---------------------|-----------------|--------------------------------------------------|------------------------------------------------|-----------------------------|---------------------------|-----------------|-----------------------|
|                 |                                           |             |               |               |       | PER CENT ERROR       | MILLIGRAMS PER CENT |                 |                                                  |                                                |                             |                           |                 |                       |
| 5               | Same Lot as No. 3                         | 38° C.      | Dark Room     | 72 hr.        | Blue  | - 1.05               | 1.029               | 9               | 0.102                                            | 0.968                                          | 1.070                       | 93.0                      | +2.88           | Late 2nd              |
| 5               | Same Lot as No. 3                         | 38° C.      | Dark Room     | 7 days        | Green | + 0.09               | 1.050               | 10              | —                                                | 1.029                                          | 1.029                       | 98.9                      | -1.05           | Early 3rd             |
| 6               | Same Lot as No. 3                         | 38° C.      | Dark Room     | 24 hr.        | Blue  | -46.25               | 0.559               | 6               | 0.409                                            | 0.614                                          | 1.023                       | 59.0                      | -1.63           | Early 2nd             |
| 6               | Same Lot as No. 3 and 5                   | 38° C.      | Dark Room     | 48 hr.        | Green | -26.63               | 0.763               | 7               | 0.256                                            | 0.790                                          | 1.046                       | 75.9                      | +0.57           | Middle 2nd            |
| 6               | Same Lot as No. 3 and 5                   | 38° C.      | Dark Room     | 72 hr.        | Blue  | -15.57               | 0.878               | 8               | 0.170                                            | 0.889                                          | 1.059                       | 85.4                      | +1.82           | Middle 2nd            |
| 6               | Same Lot as No. 3 and 5                   | 38° C.      | Dark Room     | 7 days        | Green | - 9.81               | 0.938               | 9               | 0.085                                            | 0.995                                          | 1.080                       | 95.6                      | +3.84           | Late 2nd              |
| 7               | Same Lot as No. 3 and 5                   | 38° C.      | Dark Room     | 2 hr.         | Blue  | -10.67               | 0.617               | 5               | 0.502                                            | 0.546                                          | 1.048                       | 52.5                      | +0.76           | Early 2nd             |
| 7               | HNO <sub>3</sub> Fresh Ultraviolet 1 min. | Room        | Lab. Daylight | 18 hr.        | Green | +10.09               | 1.105               | 10              | —                                                | 1.046                                          | 1.046                       | 100.5                     | +0.57           | 3rd                   |
| 7               | HNO <sub>3</sub> Fresh Ultraviolet 1 min. | Room        | Lab. Daylight | 2 hr.         | Blue  | - 4.23               | 0.996               | 8               | 0.162                                            | 0.885                                          | 1.047                       | 85.0                      | +0.70           | Middle 2nd            |
| 8               | HNO <sub>3</sub> Fresh Ultraviolet 2 min. | Room        | Lab. Daylight | 4 hr.         | Green | + 4.23               | 1.094               | 10              | —                                                | 1.046                                          | 1.052                       | 101.1                     | +1.15           | 3rd                   |

of the color change was determined by that point at which the maximum blue intensity was reached. The green color in all phases of the oxidation reaction was recorded in terms of both yellow and blue which permitted a study of the relationship of shade to the ratio existing between the two primary colors. It was then possible to determine the percentage of oxidized bilirubin both by the increase in the blue intensity and by a decrease in the yellow. A few typical experiments are reported.

*Experimental Data.*—The characteristic color change which takes place when pure bilirubin is dissolved in a simple oxidizing solution is due to its combination with oxygen from either the solution or the surrounding atmosphere. The speed with which the reaction takes place depends upon the oxidizing agent and a definite relationship exists between the reaction velocity and the availability of the oxygen. The oxidizing solutions which furnish this element in a readily available form bring about the color change without delay, while the more stable reagents which release the oxygen slowly or with difficulty produce the color transformation at a correspondingly retarded rate.

The observations on nitric acid were especially significant since it has been the reagent of choice in the quantitative estimation of bile pigment. Although it is a powerful oxidizing agent, the oxygen may not always be in a readily available form, a fact which explains the extremely wide variations found in the oxidation reaction time with bilirubin. The amount of oxygen which is free to unite with the pigment depends upon the decomposition of the acid which liberates variable quantities of nascent oxygen and nitrogen dioxide on long-standing, with heat or in sunlight. Consequently, these three factors control the reaction velocity. A specimen of nitric acid which was partially decomposed by standing in the laboratory for about a year, when made up in the usual acid alcohol mixture developed the "characteristic blue green" color in fifteen minutes. This is shown by the first solution in Table I which had actually undergone 95.1 per cent oxidation in that time. In contrast, strictly fresh nitric acid always required more than a week to complete the oxidation reaction as shown by Solutions 3 and 4, which were only 72.4 and 79.5 per cent, respectively, oxidized in seven days. Different specimens of ordinary nitric acid produced various results in the reaction time, depending upon the degree of decomposition of the acid, but always ranging within the extreme limits of fifteen minutes on the one side to a week on the other. Some specimens produced the "characteristic blue green" color in from twelve to eighteen hours as reported by Hooper and Whipple,<sup>1</sup> while others required from eighteen to twenty-four hours as given by Rous and McMaster.<sup>3</sup> Most specimens, however, brought about the color alteration at an entirely different velocity, *characteristic of the particular type of nitric acid.*

By comparing the typical results recorded for Solutions 2 and 3, it may be noted that ordinary daylight in the laboratory had no effect in hastening the color change, whereas heat increased the reaction by facilitating the decomposition of the nitric acid as shown by Solutions 3, 5, and 6. Solutions 5 and 6 incubated at 38° C. were 64.6 and 59.0 per cent, respectively, oxidized in

twenty-four hours, whereas Solution 3, as a control at room temperature, was only 32.9 per cent oxidized in the same length of time. This gives an increase in the reaction velocity of 96 and 79 per cent respectively due to increased temperature. Certain specimens of nitric acid produced the complete oxidation of the bilirubin in four hours at 38° C. as reported by Riegel, Johnston, and Ravdin.<sup>5</sup> At that temperature, however, strictly fresh nitric acid always required more than three days to complete the color change, thus emphasizing that heat is only one of the three determinative factors in bringing about the color transformation by the liberation of oxygen from nitric acid.

Irradiation of the acid alcohol mixture with ultraviolet light produced the same changes in its reaction time with bilirubin as did sunlight, heat, or long-standing. Preliminary observations showed that the nitric acid was the only constituent in the acid alcohol mixture that was influenced by this treatment. Bilirubin, however, was rapidly decolorized by irradiation, making it necessary to observe every precaution in preventing the light rays from coming in contact with the pigment, and therefore treatment of the acid alcohol mixture was carried out in another room. With ultraviolet light, it was possible to regulate at any desired speed the oxidation reaction velocity of fresh nitric acid with bilirubin. Irradiation of the acid alcohol for ten minutes brought about the complete color change in four minutes, but at this velocity a lowering of the maximum blue intensity occurred from loss of pigment by rapid oxidation. The time of exposure was gradually decreased until it was found that treatment of the acid alcohol for three minutes produced the maximum blue intensity in forty-five minutes with no appreciable loss of pigment, while the same solution without irradiation required more than seven days. Light treatment for two minutes developed the complete color change in four hours and for one minute in eighteen hours, showing a direct relationship between the length of time the acid alcohol mixture was exposed to ultraviolet light and the reaction velocity of the oxidation of bilirubin.

Comparison of the curves in Chart 1, which records the development of the blue intensity by the oxidation of bilirubin with nitric acid after one, two, and three-minute exposure of the acid alcohol mixture to ultraviolet light, showed that the contour was essentially the same as curves recording the effects of heat or long-standing on the same solution. The time required to complete the oxidation reaction was the only variation, a fact which is illustrated by superimposing the control curve, plotted in hours instead of minutes, over a curve having a more rapid velocity accelerated by irradiation (Chart 1). In all instances, the oxidation reaction velocity was comparatively rapid early in the process, becoming slower as the supply of available oxygen diminished. Calculations made from the data recorded for Solution 3, Table I, show that the strictly fresh nitric acid used in this solution accomplished 45.5 per cent of the total oxidation during the first twenty-four hours, and 14.4 per cent in the second twenty-four hours with an average of 8 per cent per day for the remainder of the week. When the nitric acid was sufficiently decomposed with ultraviolet light, as shown in Curve 3, Chart 1, the supply

of oxygen set free was sufficient to bring about the complete color change without delay, whereas when the oxygen was released slowly by the more stable specimens of nitric acid, complete oxidation of the bilirubin was greatly prolonged. Insufficient decomposition of the acid by irradiation, formed the blue intensity early with increased speed, carrying the first portion of the curve relatively high, depending upon the amount of liberated oxygen, which as it became exhausted, checked the velocity of the reaction, causing the blue intensity to rise much more slowly later, as shown by Curves 1 and 2. Regardless of the degree of decomposition of the nitric acid, the trend of the curve was always progressively upward until the point of maximum blue intensity was reached.

These observations on the development of the blue intensity are of special interest in relation to the various shades of green formed. For purposes of description one may divide the complete oxidation reaction of bilirubin into four arbitrary phases, based upon the value of the blue in-

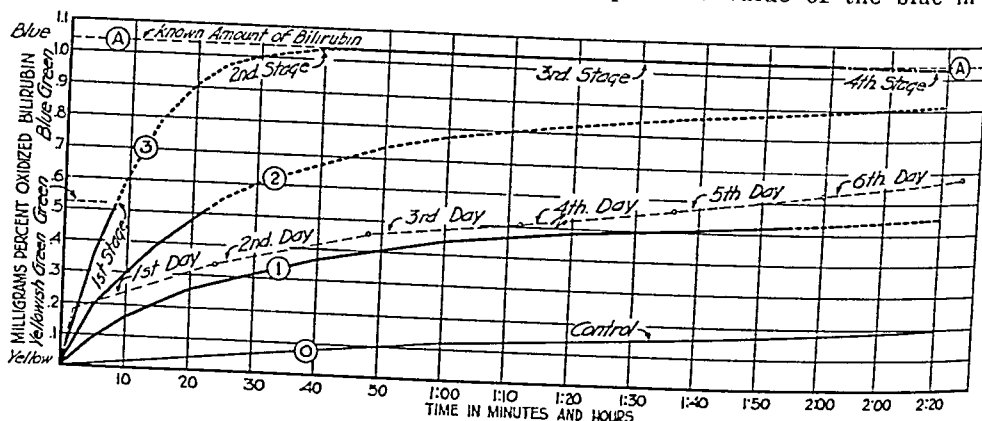


Chart 1.—Illustrating in Curves 1, 2 a development of the blue color which have been exposed to ultraviolet light for one, two, and three minutes, respectively. Each curve is divided into different stages: The first stage, which is represented by the heavy solid line, is the yellowish green phase, during which the yellow intensity predominates over the blue; the second stage, which is represented by the heavy broken line, is the blue green phase, during which the blue intensity predominates over the yellow; the third stage is the steel blue, during which the blue color remains at its maximum; the fourth stage, which is represented by the heavy dot and dash line is the phase during which the blue gradually fades out. The light solid line, O, shows the oxidation of bilirubin by the control or untreated acid alcohol mixture; the light dash and circle curve is marked off in hours instead of minutes and represents the slow oxidation of the control solution over a period of six days.

tensity as an index to the extent of the oxidation: the first, comprising all the various shades of yellowish green, begins with the oxidation of the first molecule of bilirubin and ends when 50 per cent of the pigment has been oxidized; the second, consisting of all the various shades of blue green, begins as a continuation of the first stage and ends at the point of maximum blue intensity, indicating complete oxidation of the bilirubin; the third represents that portion of the oxidation reaction during which the blue intensity remains maximum; the fourth, as shown at the end of Curve 3, illustrates the gradual fading out of the blue color (Chart 1). The color change as defined in this paper comprises the first two stages, since the third is constant and the fourth stage is characterized by a fading out of the blue color. The "characteristic

blue green" color described by Hooper and Whipple<sup>1</sup> appears early in the second stage, long before the maximum blue intensity is reached, an observation also made by Riegel, Johnston and Ravdin.<sup>5</sup> Since the former investigators used the intensity of the blue color during this stage as a base for their calculations in the quantitative estimation of bile pigment, one should note that consecutive quantitative estimations are not constant when made upon the blue intensity of the same solution during the blue green stage. This observation is also illustrated by Solution 8, Table I, which grossly was definitely well advanced in the blue green stage long before the initial two-hour reading was made, but showed an increase of 15.8 per cent in the blue intensity during the second two-hour period. From the foregoing observations one may conclude that the blue green color represents a stage of incomplete oxidation of the bilirubin, and quantitative estimations calculated upon the basis of the blue intensity only, do not represent the total pigment content because the unoxidized bilirubin remains unrecorded.

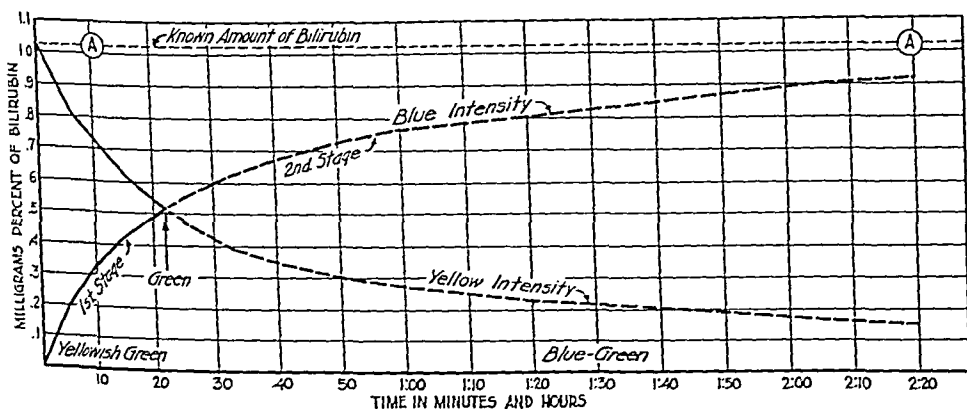


Chart 2.—Illustrating the quantitative relationship between the yellow and blue intensities in the same green solution during the color change produced by the progressive oxidation of pure bilirubin with nitrohydrochloric acid alcohol mixture. The heavy solid line shows the first stage, during which the yellow is greater than the blue and the various shades of yellow green appear. The rapid fall in the yellow was quantitatively compensated for by an equally rapid rise in the blue, so that the two curves met when exactly one-half of the bilirubin was oxidized. The heavy dashed line which represents the second stage in the oxidation of bilirubin begins at the point where the two curves cross or at the point where the blue begins to progressively predominate over the yellow and forms the various shades of blue green.

In order to understand more fully the significance of the various shades of green formed by the oxidative process, studies on the yellow intensity were a necessary counterpart to the studies on the blue. Companion quantitative studies were therefore made of both the yellow and the blue intensities on the same solution, as shown in Chart 2, which illustrates the typical incomplete reaction of nitric acid with bilirubin. The curve representing the quantitative value of the yellow intensity is practically an inverted mirror image of the blue and barring the slight unavoidable experimental errors in quantification could be inverted and superimposed upon the line representing its counterpart. The rapid fall in the yellow intensity was quantitatively compensated for by an equally rapid rise in the blue, so that these two curves crossed when exactly half of the bilirubin had been oxidized, indicating that yellow and blue are the only primary colors involved in the oxidation reaction.

This observation agrees with the statement made earlier in this paper that green is a secondary color composed of two primary colors, yellow and blue. The solution was a perfect deep green color at the point at which the curve for the yellow intensity crossed that of the blue, for at this point the values of the two intensities were colorimetrically equivalent. Up to this point, which marks the end of the first stage, the yellow intensity quantitatively predominated over the blue and the solution developed a variety of yellowish-green shades subject to the extent of the oxidation. Following the point of pigment equilibrium which was marked by the perfect green color, the blue became more and more dominant over the yellow as the oxidation progressed, developing an apparently even greater variety of blue green shades because of the slower reaction velocity during the second stage. Due to rapid oxidation early in the reaction, the first stage lasted only twenty-two minutes, whereas the second occupied several hours, finally reaching the stage of maximum blue intensity when the last traces of yellow had disappeared from the solution.

The relation of the predominant primary color to the recessive one depends upon the extent of the oxidation and control the exact shade of green developed throughout the oxidation reaction of bilirubin. By a study of the data presented in Table II, one finds this relationship to be an inverse ratio, which explains how gentle oxidation through a gradual fall in the yellow intensity and a compensating rise in the blue insidiously changes one shade of green to another. Theoretically, the shade of green is never twice the same during the continuous reaction. One can readily see that unless the reaction velocity of each solution were exactly the same, the shades would be different. This explains the difficulties encountered when matching green bile pigment solutions against one another in the colorimeter. It also explains why a series of green standards with the primary colors mixed in an inverse ratio are essential for accurate colorimetric estimation of all the possible shades of green that may be developed.

By quantitatively estimating the components of the green color separately and combining the results as recorded in Table II, one finds that the estimated value of the yellow intensity plus the blue corresponds closely to the known amount of pigment in the initial solution, a finding of practical importance in the quantitative estimation of bile pigment. With this method, regardless of the shade of color or the extent to which the bilirubin is oxidized, providing it has not yet passed the stage of maximum blue intensity, the pigmentary value of the solution can be accurately estimated at any time during the oxidation reaction. The experimental error, however, was increased slightly at the beginning and at the end of the color change, probably due to the physical difficulty of reading small amounts of yellow in a predominantly blue solution and vice versa, whereas no difficulty was encountered where the yellow to blue ratio was more equalized.

The importance of considering the yellow intensity in the quantitative estimation of bile pigment is reflected in Table I by a comparison of the experimental error recorded after estimating solutions with a known pigment content, both by the older method and by those used in this investigation.



TABLE II  
PROGRESSIVE OXIDATION OF A 1.024 MILLIGRAMS PER CENT SOLUTION OF PURE BILIRUBIN WITH THE NITROHYDROCHLORO ACID ALCOHOL MIXTURE AS THE OXIDIZING SOLUTION

| TIME.<br>P.M.<br>1/26/33 | VISIBLE COLOR   | STAGE OF<br>OXIDATION | STAND.<br>NO. | YELLOW INTENSITY                                             |        | BLUE INTENSITY |                                                    | BILIRUBIN<br>PLUS<br>BILIGYANIN | PER CENT<br>OF<br>OXIDATION | PER CENT<br>ERROR |
|--------------------------|-----------------|-----------------------|---------------|--------------------------------------------------------------|--------|----------------|----------------------------------------------------|---------------------------------|-----------------------------|-------------------|
|                          |                 |                       |               | ESTIMATED UNOXIDIZED<br>BILIRUBIN CORRECTED FOR<br>1 MINUTE* |        | TIME<br>P.M.   | ESTIMATED<br>OXIDIZED<br>BILIRUBIN<br>(BILIGYANIN) |                                 |                             |                   |
| 1:16                     | Yellowish green | Early 1st             | 2             | 0.811                                                        | 0.035* | 1:15           | 0.201                                              | 1.047                           | 19.6                        | 2.27              |
| 1:21                     | Yellowish green | Middle 1st            | 3             | 0.712                                                        | 0.019  | 1:20           | 0.333                                              | 1.064                           | 32.5                        | 3.97              |
| 1:26                     | Yellowish green | Middle 1st            | 4             | 0.604                                                        | 0.021  | 1:25           | 0.424                                              | 1.049                           | 41.4                        | 2.48              |
| 1:31                     | Yellowish green | Late 1st              | 5             | 0.530                                                        | 0.015  | 1:30           | 0.488                                              | 1.033                           | 47.6                        | 0.87              |
| 1:36                     | Green           | Onset 2nd             | 5             | 0.465                                                        | 0.013  | 1:35           | 0.547                                              | 1.025                           | 53.4                        | 0.09              |
| 1:43                     | Green           | Early 2nd             | 6             | 0.384                                                        | 0.010  | 1:42           | 0.619                                              | 1.023                           | 60.4                        | 0.09              |
| 1:50                     | Bluish green    | Early 2nd             | 6             | 0.354                                                        | 0.008  | 1:49           | 0.664                                              | 1.023                           | 64.8                        | 0.19              |
| 2:00                     | Bluish green    | Middle 2nd            | 7             | 0.304                                                        | 0.009  | 1:59           | 0.721                                              | 1.034                           | 70.4                        | 0.97              |
| 2:10                     | Blue green      | Middle 2nd            | 7             | 0.268                                                        | 0.005  | 2:09           | 0.763                                              | 1.036                           | 74.5                        | 1.19              |
| 2:20                     | Blue green      | Middle 2nd            | 8             | 0.254                                                        | 0.001  | 2:19           | 0.778                                              | 1.033                           | 75.9                        | 0.87              |
| 2:30                     | Blue green      | Middle 2nd            | 8             | 0.226                                                        | 0.002  | 2:29           | 0.803                                              | 1.031                           | 78.4                        | 0.68              |
| 2:40                     | Blue green      | Middle 2nd            | 8             | 0.219                                                        | 0.0007 | 2:39           | 0.821                                              | 1.0407                          | 80.1                        | 1.64              |
| 2:52                     | Blue green      | Middle 2nd            | 8             | 0.203                                                        | 0.001  | 2:51           | 0.852                                              | 1.056                           | 83.1                        | 3.11              |
| 3:01                     | Blue green      | Middle 2nd            | 9             | 0.180                                                        |        | 3:00           | 0.871                                              | 1.051                           | 85.0                        | 2.68              |
| 3:16                     | Blue green      | Late 2nd              | 9             | 0.161                                                        |        | 3:15           | 0.902                                              | 1.063                           | 88.0                        | 3.80              |
| 3:30                     | Blue green      | Late 2nd              | 9             | 0.148                                                        |        | 3:29           | 0.920                                              | 1.068                           | 89.8                        | 4.38              |

\*Calculated as the average decrease per minute for the preceding five minutes.

For example, when Solution 3 was estimated in twenty-four hours, by the copper sulphate and potassium bichromate standard with Filter No. 72, which records the intensity of the blue color only, the estimated pigmentary value was found to be 0.320 mg. per cent, giving an error of 69.23 per cent. In contrast, when compared in the colorimeter with standard No. 3, the same solution was found to be 32.7 per cent oxidized with a yellow and blue concentration of 0.692 mg. and 0.341 mg. per cent respectively, giving a total pigment content of 1.033 mg. per cent, with an experimental error of 0.67 per cent. This table illustrates that the yellow intensity in the green solutions which corresponds to the unoxidized bilirubin fraction if ignored is recorded as a part of the experimental error.

The observations on the green color formed by the oxidation of bilirubin made during this investigation differ somewhat from the usual impressions given in the textbooks. It is generally implied that biliverdin is the direct oxidation product of bilirubin. These experiments do not entirely contradict this, but tend to explain the manner in which the color of biliverdin is actually formed. The total oxidation of bilirubin completely transformed the yellow solution to a blue color commonly known as bilieyanin, while the partial oxidation formed a great variety of green shades, depending upon the extent of the oxidation. Based upon the present scientific classification of colors, these studies indicate that biliverdin is a mixture of bilirubin and bilieyanin rather than a definite entity. Coordinate quantitative studies on both the yellow and the blue intensities in the same solution illustrate that the oxidation reaction which was characterized by a gradual and progressive shifting in color from yellow through green to blue was accompanied by a quantitative transformation of bilirubin to bilieyanin.

#### SUMMARY

Stimulated by difficulties encountered in the colorimetric methods for the quantitative estimation of bile pigments, a study of the quantitative color change through all phases of the oxidation of bilirubin has been made. The factors influencing the reaction velocity of the oxidation of bilirubin, and the factors controlling variations in the resultant shades of green, yellow green and blue green have been investigated by the preparation and use of a series of suitable standards which are composed of definite mixtures of stable blue and yellow dyes.

It was found that the time required to complete the color change from yellow to deep blue varied with the specimens of nitric acid employed which in turn differed in their supply of free oxygen. External factors such as heat, light, and ultraviolet irradiation influenced the rate of oxidation of bilirubin by virtue of their direct effect upon the liberation of free oxygen from the nitric acid. The blue green color generally employed in colorimetric determinations represents a stage in the incomplete oxidation of bilirubin, and hence determinations based upon this method do not quantitatively represent the amount of bile pigment present in the solution. However, by the quantitative estimation of both the blue and the yellow components, with the aid of light filters and suitable dichromatic standards, the total amount of pigment

in the original solution can be accurately determined at any stage in the oxidation reaction. These experiments indicate that the bile pigment generally designated as biliverdin is a mixture of bilirubin and its oxidation product bilieyanin.

The authors take great pleasure in expressing their sincere appreciation to Icie G. Maey, Ph.D., for her generous aid during the course of this work.

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## DEMONSTRATION OF PENETRATION OF AN ANTISEPTIC DYE INTO THE TISSUES OF THE GENITOURINARY TRACT\*

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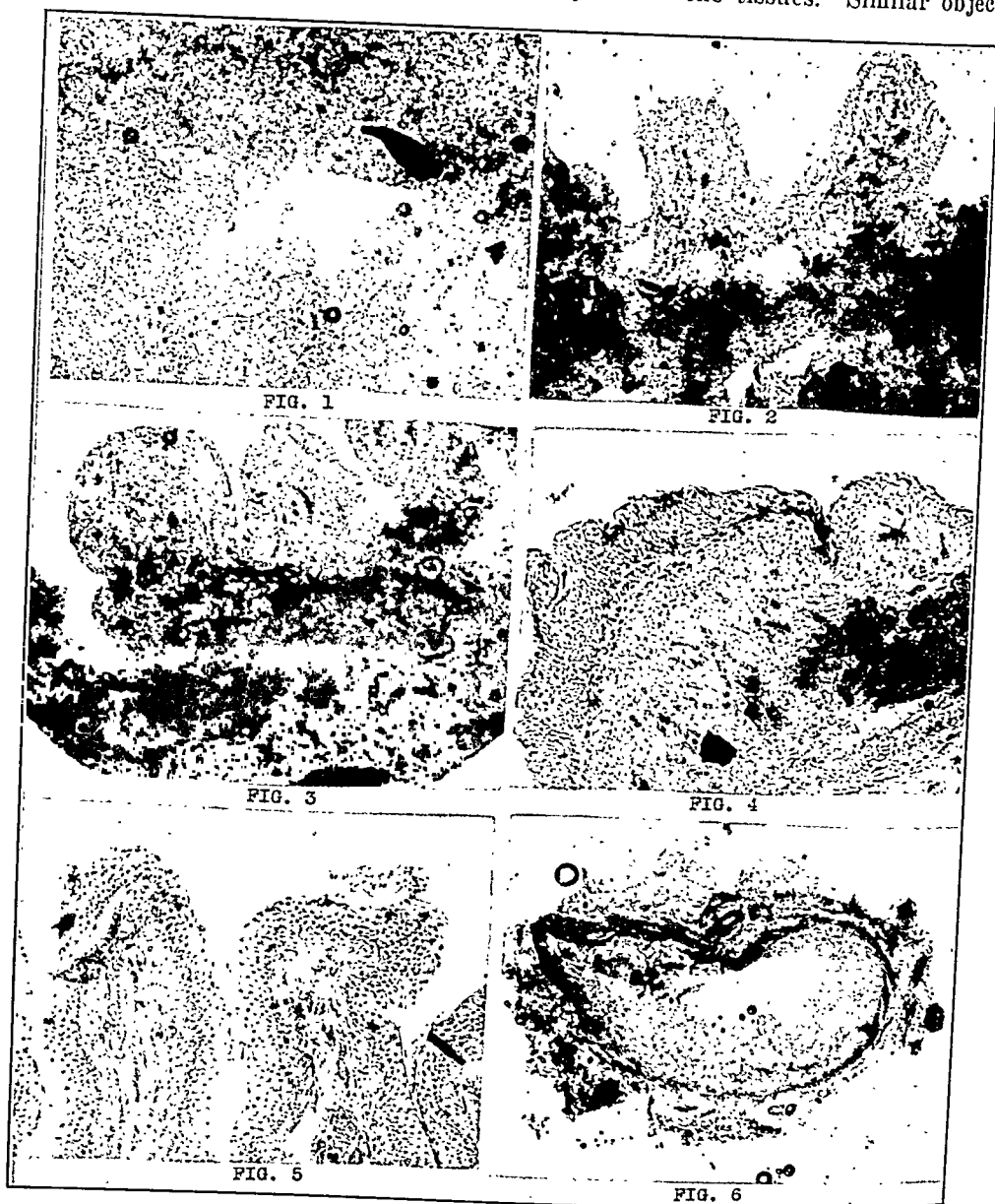
IN VIEW of the fact that the effective results of a urinary antiseptic may be conditioned not only by concentration in which it is bacteriostatic and bactericidal, but also by the ability of the compound to penetrate into the tissues, it was considered to be of some interest to demonstrate such penetration into the tissues lining the genitourinary tract.

Experiments to show such penetration were carried out as follows: Irrigations of urethra and bladder were made on a series of male rabbits, using a 0.5 per cent solution of the dye. This was supplemented by injections into the pelvis of the kidney via ureter before removal of the entire genitourinary tract. The dye used for these experiments was phenyl-azo-diamino-pyridine hydrochloride (pyridium).

In the preparation of histologic sections, difficulty was encountered in preventing the dye from being leached out of the tissues by the various agents used for fixation, hardening, imbedding, and clearing. After numerous experiments with different procedures, it was found that the most satisfactory method was one in which the tissues were fixed in mercuric chloride solutions

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and then frozen sections cut, mounted, and immediately photographed. Although other metallic salts also are capable of precipitating the dye, all were found to be more or less unsatisfactory due to the fact that subsequent manipulations of the section leached out the dye from the tissues. Similar objec-



tions were encountered in the case of the alkaloidal reagents, such as picric acid and Lugol's solution, which precipitate the dye, or in the case of alkalis which precipitate the free base.

Mercuric chloride was finally selected since it produced the least soluble, as well as the most highly colored precipitate even in fairly dilute solutions. Before making this selection various other standard fixatives were tried, but

difficulty was always encountered either because the dye was not precipitated or was in fact leached out of the tissues by the fixing solution. Although mercuric chloride was found to be the best precipitant and fixative ordinary imbedding processes involving the use of alcohol and alcohol-ether mixtures for purposes of dehydration were found to bring about solution and diffusion of the mercuric compound of the dye into other nonstained parts of the tissues. The most suitable method for demonstration of the true extent of penetration was found to be as follows: Small portions of excised tissue are placed in bichloride solution for twenty-four hours, frozen sections are then cut, the sections floated in bichloride solution, mounted in glycerin, and photographed.

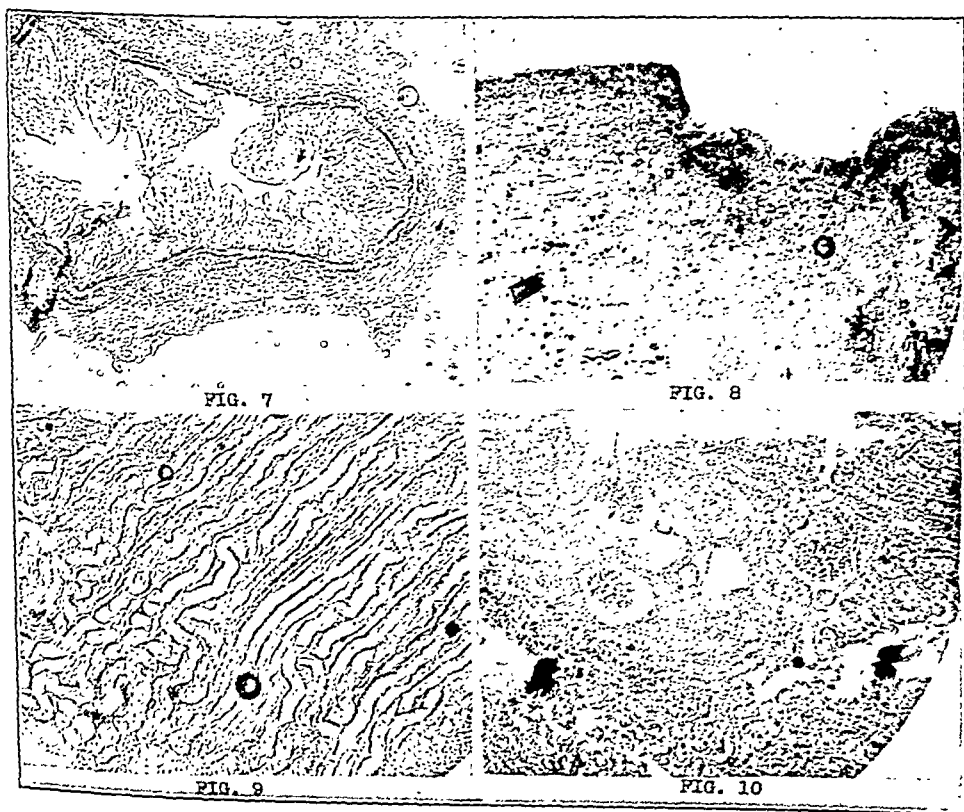


Fig. 1 is a cross-section of the urethra under low power magnification and shows the mercuric precipitate of the dye. Figs. 2 and 3 are sections of bladder wall under low power magnification showing precipitated dye in the tissues. Fig. 4 is also a cross-section of the bladder wall under high power magnification and may be compared with Fig. 5 which is a similar section of normal bladder wall. Fig. 6 is a cross-section of the ureter showing precipitate of the dye and should be compared with Fig. 7, a section of normal ureter. Fig. 8 is a section of kidney tissue adjacent to the pelvis of this organ and shows dye precipitate, whereas Figs. 9 and 10 are sections of deeper portion of the kidney and show no penetration.

## SOME EXPERIMENTAL STUDIES ON NEMBUTAL (PENTO-BARBITAL-SODIUM)<sup>2</sup>

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IN TESTING a constant pressure apparatus, with which we wished to be able to inject glucose solutions intravenously at a definite rate over long periods of time, it was found convenient to employ an anesthetic. Nembutal was selected, because of its rapidity of action, and shorter acting time than the other barbiturates. Urine was to be collected and tested for reducing substances. It was observed that very little urine was obtainable during the period of narcosis.

Fee<sup>1</sup> has shown that water diuresis established in decerebrate dogs, by the administration of water through the stomach tube, is checked by the administration of chloroform, ether, chloralose, or morphine in doses commonly employed to produce anesthesia, and the inhibition lasts, roughly, for the same length of time as the narcotic effect.

Frey,<sup>2</sup> in his experiments on rabbits, using urethane, chloral hydrate, and morphine, found that diuresis was decreased whether the water was administered by mouth, by rectum, or through an intestinal fistula.

Ogden<sup>3</sup> demonstrated the inhibition of water diuresis by amytal, and Marx<sup>4</sup> concluded that amytal had a strong antidiuretic effect on dogs, and moreover may cause a retention of substances in the blood ordinarily excreted through the kidneys.

Swanson and Shonle,<sup>5</sup> in their pharmacologic study of nembutal (pento-barbital-sodium), pointed out the fact that nembutal is an isomer of amytal, the difference being in the alkyl side chain, the methyl group in the amytal molecule being attached to the  $\gamma$ -c-atom of the side chain, while in the iso-amytal molecule the methyl group is linked to the  $\alpha$ -c-atom. The position of this methyl group seemingly has a great influence on the physiologic activity.

The disputed question as to the effect of amytal on blood sugar<sup>6, 7, 8, 9, 10</sup> raised the question as to the effect of pento-barbital-sodium on the blood sugar level of a fasting animal.

Hines, et al.,<sup>11</sup> showed that a greater hyperglycemia and a marked increase of glycosuria existed when glucose solution was injected intravenously under amytal anesthesia than in the control experiments on the same animal; whereas Brill<sup>12</sup> concluded that sodium amytal had a marked inhibiting effect on the hyperglycemia produced by morphine.

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## EXPERIMENTAL

Five mongrel dogs in normal condition, so far as could be determined by blood and urinary laboratory methods and physical examination, were used.

Each dog received 1 c.c. (1 c.c. = 1 gr.) nembutal (pento-barbital-sodium, Abbott) per 5 pounds body weight. The dogs varied from 14 to 27 pounds in weight. The dogs were fed their usual kennel rations at 2:00 P.M. and received no feeding other than water, which was always accessible. They were accustomed to the laboratory and any excitement was avoided as much as possible.

The next morning, about sixteen or eighteen hours after last food given, they were brought into the laboratory, and 3 c.c. of blood was obtained by venous puncture of the saphenous vein, and oxalated to prevent clotting. Serum was obtained by allowing 2 c.c. of blood to clot.

Water ranging from 200 c.c. to 300 c.c., depending on the size of the animal, was given by stomach tube. The bladder was emptied immediately, and emptied at twenty-minute intervals thereafter, and the amount measured and recorded. Normals for urinary output under water diuresis, blood sugar, urea, nonprotein nitrogen, and total serum protein were obtained for each dog.

The blood samples were secured at approximately hourly intervals, and until such time as the animals indicated that they were reacting well from the anesthesia as manifested by attempts to raise head, stand, etc.

The nembutal was injected intravenously at such a rate that the animals were unconscious at the termination of the injection. During the injection, the respiration always quickened. Injecting too rapidly produced a rapid, jerky respiration. The respiration became slow, full, and deep as soon as the animals were unconscious. There never was any period of excitement, and the same results were always obtained on subsequent injections on the same animal.

On the dosage given, 1 c.c. per 5 pounds (1 c.c. = 1 gr.), a surgical anesthesia was obtained in about five minutes and persisted from forty-five to fifty minutes. The dogs remained asleep a variable length of time, but all were showing definite signs of awakening within three and a half hours and usually were on their feet in four to four and one-half hours with only slight ataxia and incoordination persisting.

The normal urinary output, and the output under nembutal anesthesia, have been charted (Chart 1) for each dog to show the decrease in the urinary output.

Only one record for each dog is shown to avoid confusion on the chart, but several records on each were made, and all responded in a like manner. Increasing the dosage of nembutal, as little as 0.5 c.c. resulted in a greater decrease in the urinary output. In Dog 17, increasing the dosage from 3 c.c. to 3.5 c.c. resulted in anuria during the period of narcosis.

With an increase in the total dosage of nembutal a longer period of anesthesia resulted, both in the period of surgical anesthesia and in the time in which reaction was first noted.

In only one instance (Dog 10) did an active diuresis seem to begin after the animal began to react. It is possible that during the longer time in which

narcosis is present sufficient water is lost by other routes, other than through the kidney, so that there is not sufficient excess water present to produce an active diuresis.

Marx, II.<sup>13</sup> states that amytal has an influence on the cerebral centers, regulating metabolism of water, and that it also influences the endothelium of the capillaries, changing the permeability; from which he concluded that the hypnotic directs the water to the tissues of the body in general, instead of

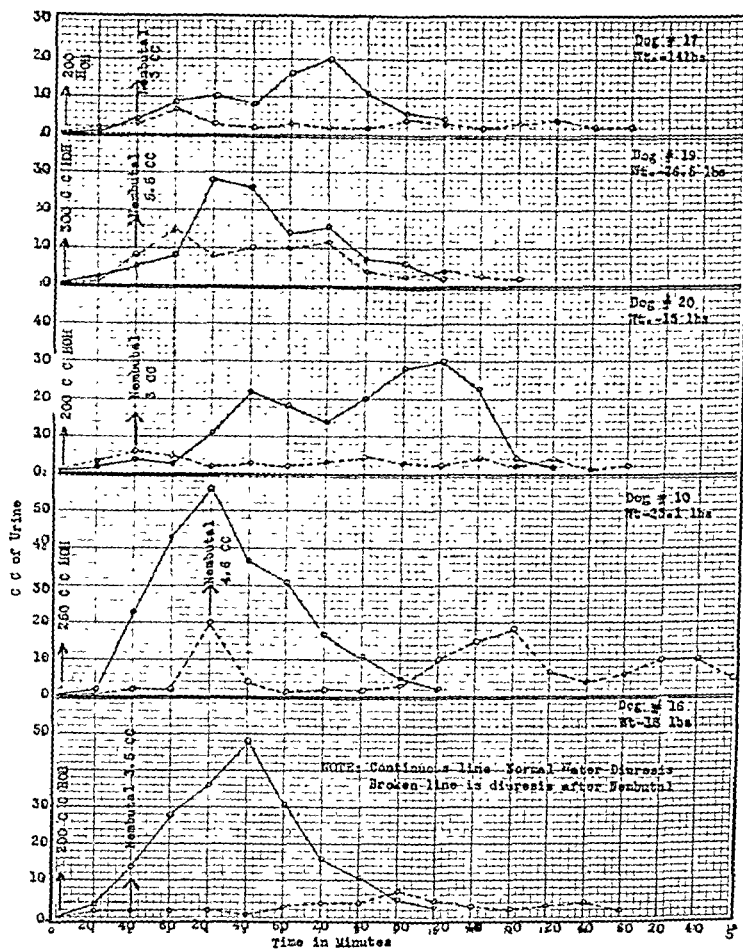


Chart 1

to the kidneys. We are inclined to believe that nembutal has a similar action. This action is not peculiar to the barbiturates alone, as it is possible to produce edema by injecting sodium chloride infusions quite readily, if chloroform, ether, or chloral hydrate has been used to anesthetize the animal.

From Pratt's<sup>14</sup> work, one can infer that the liver breaks down and detoxifies pento-barbital, because in experimental lesions of the liver, produced by chloroform, nembutal in the usual dosage produces prolonged narcosis.

We thought it might be interesting to note the blood chemistry changes, if any, during the period of narcosis, and if repeated injections would result in damage to the liver and kidneys.



Since the blood chemistry studies were in agreement with each dog tested, the results of the study of one dog only are tabulated (Table I).

TABLE I

| TIME       | DOG 10<br>BLOOD SAMPLES | SUGAR—NORMAL | SUGAR DURING<br>NEMBUTAL AN-<br>ESTHESIA | UREA—NORMAL | UREA DURING<br>NEMBUTAL AN-<br>ESTHESIA | T.S.P.—NORMAL | T.S.P. DURING<br>NEMBUTAL<br>ANESTHESIA | N.P.N.—NORMAL | N.P.N. DURING<br>NEMBUTAL<br>ANESTHESIA |
|------------|-------------------------|--------------|------------------------------------------|-------------|-----------------------------------------|---------------|-----------------------------------------|---------------|-----------------------------------------|
| 10:00 A.M. | First sample            |              | 78.7                                     |             | 20.4                                    |               | 5.2                                     |               | 23.6                                    |
| 10:20      | Second sample           | 64.5         | 103.2                                    | 19.1        | 22.6                                    | 5.4           | 4.8                                     | 25.2          | 20.8                                    |
| 11:20      | Third sample            | 64.7         | 76.2                                     | 20.6        | 21.6                                    | 5.7           | 4.53                                    | 26.2          | 18.5                                    |
| 12:20 P.M. | Fourth sample           | 74.3         | 88.5                                     | 20.3        | 21.4                                    | 5.9           | 4.41                                    | 26.4          | 23.2                                    |
| 1:20       | Fifth sample            | 76.2         | 90.5                                     | 20.3        | 21.8                                    | 5.7           | 4.71                                    | 26.1          | 24.2                                    |

There was in each instance a slight rise in the blood sugar levels, though not sufficient in our opinion to warrant the conclusion that nembutal raises the blood sugar level. The rises noted might be accounted for by the metabolic changes occurring during that length of time.

Because of the interest and controversy in regard to the effect of amytal on the blood sugar level, the results of the five dogs studied will be tabulated below (Table II).

The blood samples were obtained as follows:

- Sample 1, before giving water
- Sample 2, 20 minutes after giving nembutal
- Sample 3, 1 hour after second sample
- Sample 4, 1 hour after third sample
- Sample 5, 1 hour after fourth sample

TABLE II

## BLOOD SUGAR DURING NEMBUTAL ANESTHESIA

|               | DOG 16 | DOG 10 | DOG 20 | DOG 19 | DOG 17 |
|---------------|--------|--------|--------|--------|--------|
| First sample  | 55.5   | 78.7   | 57.8   | 85.1   | 58.3   |
| Second sample | 58.2   | 103.2  | 66.2   | 93.4   | 68.9   |
| Third sample  | 71.8   | 76.2   | 83.4   | 94.3   | 64.7   |
| Fourth sample | 74.1   | 88.5   | 83.5   | 71.2   | 64.1   |
| Fifth sample  | 66.2   | 90.5   | 75.6   | 71.5   | 63.5   |

In one of our experiments, in which we were injecting a glucose solution, 6 per cent, intravenously, we found the blood sugar level to rise to 256 mg. per 100 c.c., without producing any diuresis. While further experimentation on this point is necessary, it indicates that the inhibition of water diuresis, produced by nembutal, cannot be broken by infusions of glucose at such a rate that the renal threshold is exceeded.

Three of our dogs have received repeated injections (1 c.c. per 5 pounds [1 c.c. = 1 gr.]) at intervals of six days. Dog 16 has had 8 injections and dogs 10 and 19 ten such injections. The animals did not lose weight and their blood chemistries and urinalyses were within normal limits one week after the last injection.

## TO SUMMARIZE

1. Nembutal seems to be an efficient and rapidly producing anesthetic, with no initial period of excitement, with a shorter period of narcosis, and a relatively shorter reacting time than the other barbiturates.

2. Water diuresis in dogs is inhibited by doses of nembutal (pento-barbital-sodium, Abbott), sufficient to produce anesthesia. (Dose: 1 gr. per 5 pounds body weight.) In one of our dogs, increasing the dosage, a complete suppression of urine followed, during the period of narcosis.

3. There is a slight change in the blood sugar during the period of narcosis.

4. Blood chemistry studies, including urea, nonprotein nitrogen, and total serum protein, were not appreciably affected during the period of narcosis.

5. Three dogs, receiving as many as 8 to 10 injections at intervals of six days, showed no changes in hepatic or renal functions that could be detected by the usual blood and urine laboratory examinations.

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# THE USE OF HYDROGEN PEROXIDE IN THE QUANTITATIVE ESTIMATION OF BILIRUBIN IN BILE\*

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IN A CRITICAL study of the quantitative estimation of bile pigment,<sup>1</sup> it was shown that there are errors in the current colorimetric method<sup>2-5</sup> which are due to the inefficient and variable oxidizing agent employed and the failure to consider the unoxidized pigment fraction which forms a part of the blue green color used in making the colorimetric readings. It was demonstrated that the amount of free oxygen which controls the color change depends upon the degree of decomposition of the different specimens of nitric acid used in making up the oxidizing solution and this in turn produced extremely wide variations in its oxidation reaction time with bilirubin. Consequently, the various degrees of spontaneous decomposition existing in the nitric acid standing in the different laboratories, presents an inconstant variable which renders the results on the quantitative estimation of bilirubin incomparable. Furthermore, it was shown that the changes in the color of the pigment solution were produced by a gradual and progressive oxidation of the bilirubin to bilicyanin and that the green solution commonly known as biliverdin should be considered colorimetrically as a mixture of the two pigments, bilirubin and its oxidation product, bilicyanin, each of which must be estimated separately in order to ascertain the pigmentary value of the green solutions produced in the oxidation reaction.

In the present report an attempt has been made to eliminate the errors due to the variability of the nitric acid by replacing it with hydrogen peroxide which gives a more constant and rapidly available source of free oxygen. The difficulties as well as the errors involved in estimating the pigmentary value of the blue green color are eliminated when the readings are made later in the oxidation reaction, namely, during the stage of maximum blue intensity. The final colorimetric readings with hydrogen peroxide as the source of available oxygen in the oxidizing solution can be made within thirty minutes instead of the former from eighteen to twenty-four hours, thus giving a simplified, rapid and accurate method for the quantitative estimation of the bilirubin in bile.

## PREPARATION OF SOLUTIONS

*Stock Bilirubin Solution.*—Transfer 25 mg. of pure bilirubin† to a clean dry 100 c.c. volumetric flask and make up to volume with chloroform.

*Nile Blue Sulphate Solution.*—Transfer 15 mg. of Nile blue sulphate‡ to a 100 c.c. volumetric flask and make up to volume with 95 per cent ethyl alcohol.

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†This study was made possible by a grant and the facilities of the Research Laboratory of the Children's Fund of Michigan and the Children's Hospital of Michigan, Detroit.

‡The bilirubin was obtained from the Eastman Kodak Company, Rochester, New York.

§Nile blue sulphate (diethylaminonaphthophenoxazine) ( $C_{20}H_{22}N_2O$ ):  $SO_4$  made by The Coleman and Bell Company, Norwood, Ohio, was used.

**Oxidizing Reagent.**—A solution of 2 c.c. of concentrated hydrochloric acid and 0.4 c.c. of 30 per cent hydrogen peroxide\* is made up to 100 c.c. with 95 per cent ethyl alcohol.

#### STANDARDIZATION OF THE DYE SOLUTION

Transfer 1 c.c. of the stock bilirubin solution into each of five 25 c.c. volumetric flasks and dilute to volume with the oxidizing reagent. After thoroughly shaking allow the solution to stand for thirty minutes at room temperature until the blue color develops. Dilute 10 c.c. of the Nile blue sulphate solution to 500 c.c. with 95 per cent ethyl alcohol. Using the 1 mg. per cent solutions of oxidized bilirubin as standards determine the bilirubin equivalent of the diluted blue dye solution. The average of the five determinations should be taken as the final result. Place the Wratten Monochromatic Light Filter No. 72† over the eyepiece of the colorimeter when making the readings. Calculate the values for the dye solution by the formula as follows:

$$\frac{\text{Reading of standard} \times \text{Concentration of standard in mg. per 100 c.c.}}{\text{Reading of unknown}} = \text{Bilirubin equivalent of the blue dye in mg. per 100 c.c.}$$

**Method for the Determination of Bilirubin in Bile ‡**—Transfer 1 c.c. of the bile to be analyzed to a clean dry 50 c.c. volumetric flask and make up to volume with the oxidizing reagent. After thoroughly shaking allow the solution to stand for thirty minutes at room temperature until the blue color develops. Clear the solution of precipitated mucus in the centrifuge or by filtering and compare intensity of the cleared solution in the colorimeter with that of the stable blue standard. Place the Wratten Monochromatic Light Filter No. 72 over the eyepiece of the colorimeter before making the readings. The calculations are made as follows:

$$\frac{\text{Reading of standard} \times \text{Concentration of standard in mg. per 100 c.c.} \times \text{dilution}}{\text{Reading of unknown}} = \text{Mg. of bilirubin per 100 c.c.}$$

The experimental error of the method with known solutions of pure bilirubin is less than 2 per cent. The variation in results with bile itself, however, is increased at times to nearly 5 per cent due to the inability to deliver aliquot specimens of mucus laden fluid from the ordinary pipette. For this reason triplicate determinations should be averaged in order to reduce the experimental error to a minimum and every precaution should be exercised in uniformly draining the pipette.

\*Merck's hydrogen peroxide, 30 per cent (Superoxol).

†Wratten Monochromatic Light Filter No. 72 was obtained from the Eastman Kodak Company, Rochester, New York. The shade of the final blue color developed from fresh bile for the readings is sufficiently constant to eliminate the use of this filter but frequently the final tint is changed by the presence of other pigments in the gallbladder and this filter eliminates such interferences. Filter No. 72 permits the intensity of the blue rays only.

‡Experiments show that the principles of this method may be applied to the quantitative estimation of bilirubin in serum and urine. Further work is in progress on the details of the technic.

## DISCUSSION

Although bilirubin is the parent chromogenic substance in bile, there may be other pigmentary substances formed on standing through its oxidation, reduction, hydrolysis, or conjugation with other bile constituents. Due to the fact that bilirubin is an unstable pigment, quantitative estimations should be made upon strictly fresh bile before any spontaneous chemical changes occur to cause the loss of pigment. Because of the natural affinity of bilirubin for oxygen, which it quickly absorbs from the solution or the surrounding atmosphere, spontaneous oxidation is frequently the earliest change that occurs in fresh bile and may even take place within the gallbladder, while it invariably occurs subsequent to removal from the body and exposure to the air. Colorimetric studies indicate that bilicyanin, which is the direct oxidation product of bilirubin, is present in greater or lesser amounts in the specimens of bile which have already turned green from spontaneous oxidation. Although both

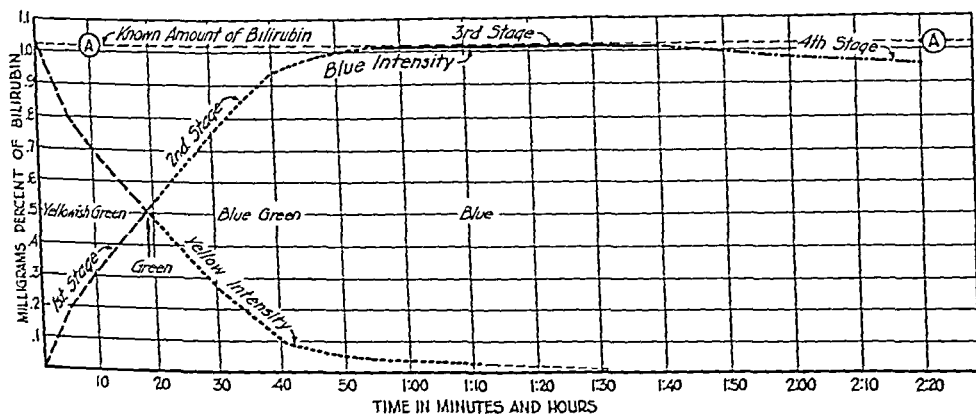


Chart 1.—Illustrating the four stages in the progressive oxidation reaction of a 1.024 mg. per cent solution of pure bilirubin with the hydrogen peroxide-hydrochloric acid-alcohol oxidizing reagent and the relationship of the two primary colors which form the green solution. The first stage is yellowish green color due to the predominance of the unoxidized bilirubin over its oxidation product, bilicyanin; the second stage is blue green color due to the bilicyanin predominating over the bilirubin; the third stage represents the maximum blue intensity developed from the bilirubin by oxidation and illustrates the most reliable position in the oxidation reaction for quantitatively estimating the total pigment content by means of a single monochromatic blue standard; during the fourth stage the blue intensity gradually fades out.

spontaneous and induced oxidation transform the bilirubin to bilicyanin, it so happens that the oxidizing reacts with both pigments so that the bilicyanin previously formed by spontaneous oxidation may be further oxidized to colorless end-products by the time the remainder of the bilirubin is oxidized to bilicyanin. Experience shows that 2 or 3 per cent of bilicyanin mixed with the bilirubin gives the solution a definite greenish tint, and yet, this amount is within the limits of the experimental error, so that even though the bile specimen is slightly greenish the simple method described above is sufficiently accurate for practical purposes. In specimens which have undergone greater spontaneous oxidation, however, it becomes necessary to make separate colorimetric estimations of the two constituent pigments, bilirubin and bilicyanin, as described in a previous publication<sup>1</sup> and illustrated in Table I.

TABLE I  
PROGRESSIVE OXIDATION OF A 1.024 MG. PER CENT SOLUTION OF PURE BILIRUBIN WITH HYDROGEN PEROXIDE HYDROCHLORIC ACID ALCOHOL

| TIME<br>4:00 P.M.<br>1/12/33 | VISIBLE<br>COLOR | STAGE<br>OF<br>OXIDATION | STANDARD<br>NO. | YELLOW INTENSITY                                   |                                   | BLUE INTENSITY |                                        | BILIRUBIN<br>PLUS<br>BILICYANIN OXIDATION | PER CENT<br>OF<br>OXIDATION | PER CENT<br>ERROR |
|------------------------------|------------------|--------------------------|-----------------|----------------------------------------------------|-----------------------------------|----------------|----------------------------------------|-------------------------------------------|-----------------------------|-------------------|
|                              |                  |                          |                 | ESTIMATED<br>BILIRUBIN<br>CORRECTED<br>FOR 1 MIN.* | ESTIMATED UNOXIDIZED<br>BILIRUBIN | TIME<br>P.M.   | ESTIMATED<br>BILIRUBIN<br>(BILICYANIN) |                                           |                             |                   |
| 4:06                         | Yellowish green  | Early first              | 2               | 0.788                                              | 0.039*                            | 4:05           | 0.190                                  | 1.017                                     | 18.5                        | 0.68              |
| 4:11                         | Yellowish green  | Middle first             | 3               | 0.672                                              | 0.023                             | 4:10           | 0.319                                  | 1.014                                     | 31.1                        | 0.97              |
| 4:16                         | Green            | Late first               | 4               | 0.571                                              | 0.020                             | 4:15           | 0.421                                  | 1.012                                     | 41.1                        | 1.17              |
| 4:21                         | Green            | Onset second             | 5               | 0.461                                              | 0.022                             | 4:20           | 0.539                                  | 1.022                                     | 52.6                        | 0.19              |
| 4:26                         | Bluish green     | Early second             | 6               | 0.359                                              | 0.020                             | 4:25           | 0.642                                  | 1.021                                     | 62.7                        | 0.29              |
| 4:31                         | Bluish green     | Middle second            | 7               | 0.254                                              | 0.021                             | 4:30           | 0.754                                  | 1.029                                     | 73.6                        | 0.48              |
| 4:36                         | Blue green       | Middle second            | 8               | 0.169                                              | 0.016                             | 4:35           | 0.852                                  | 1.037                                     | 83.2                        | 1.27              |
| 4:41                         | Blue green       | Middle second            | 9               | 0.091                                              | 0.015                             | 4:40           | 0.941                                  | 1.047                                     | 91.8                        | 2.27              |
| 4:46                         | Blue green       | Late second              | 9               | 0.074                                              | 0.003                             | 4:45           | 0.979                                  | 1.056                                     | 95.6                        | 3.11              |
| 4:51                         | Blue green       | Late second              | 9               | 0.046                                              | 0.005                             | 4:50           | 1.007                                  | 1.058                                     | 97.7                        | 3.32              |
| 4:56                         | Blue green       | Late second              | 9               | 0.041                                              | 0.001                             | 4:55           | 1.018                                  | 1.060                                     | 99.4                        | 3.51              |
| 5:01                         | Blue green       | Late second              | 9               | 0.039                                              |                                   | 5:00           | 1.018                                  | 1.057                                     | 99.4                        | 3.27              |
| 5:06                         | Blue green       | Late second              | 9               | 0.036                                              |                                   | 5:05           | 1.018                                  | 1.054                                     | 99.4                        | 2.92              |
|                              | Blue             | Third                    | 10              |                                                    |                                   | 5:10           | 1.018                                  | 1.018                                     | 99.4                        | 0.58              |
|                              | Blue             | Third                    | 10              |                                                    |                                   | 5:15           | 1.018                                  | 1.018                                     | 99.4                        | 0.58              |
|                              | Blue             | Third                    | 10              |                                                    |                                   | 5:40           | 1.017                                  | 1.017                                     | 99.3                        | 0.68              |
|                              | Blue             | Fourth                   | 10              |                                                    |                                   | 6:00           | 0.979                                  | 0.979                                     |                             | 4.49              |

\*Calculated as the average decrease per minute for the preceding five minutes.

There are four stages in the complete oxidation of pure bilirubin as shown in Chart 1 and Table I. During the first stage, the yellow unoxidized bilirubin predominates over its blue oxidation product, bilieyanin, while during the second stage their ratio is reversed and forms the blue green color generally used in colorimetric comparisons. It has been demonstrated that the blue green color represents a stage of incomplete oxidation of bilirubin, and quantitative estimations made during this stage not only require the determination of the oxidized bilirubin but also the pigment fraction that remains unoxidized.<sup>1</sup>

For such a determination it is necessary to employ the more complicated series of dichromatic green standards which contain the primary colors mixed in an inverse ratio.<sup>1</sup> In contrast, the present accurate yet simplified method for determining bilirubin utilizes the maximum blue intensity of the third stage of oxidation for the colorimetric readings and requires but a single monochromatic blue standard. As the oxidation progresses from the second into the third stage the color changes from the blue green to blue, and the curve for the blue intensity ceases to rise, remaining at the maximum level for considerable time, due to the fact that at this time the available oxygen is nearly depleted and the oxidation velocity is comparatively slow. Since the time limits of the third stage are sufficiently long to permit readings of a series of solutions without undue haste and the blue color remains constant during this stage, it offers, therefore, the most reliable period during the oxidation reaction of bilirubin in which to quantitatively estimate the total pigment content by monochromatic methods.

Through the introduction of hydrogen peroxide to replace the nitric acid in the oxidizing solution, the practicability of using the third stage for the final readings is made possible. Because of its labile oxygen, hydrogen peroxide has the ability to complete the oxidation of bilirubin to bilieyanin within thirty minutes instead of the former eighteen to twenty-four hours. The maximum blue intensity developed with hydrogen peroxide from a known amount of pure bilirubin is remarkably constant, varying only with the amount of pigment in the initial solution. Slight alterations in the amount of hydrogen peroxide do not alter the height of the maximum blue intensity but only serve to increase or decrease the length of time the blue intensity remains at its maximum. The same is true for variations in the hydrochloric acid in the oxidizing solution. In order to make accurate readings on both the yellow and blue colors in the same solution as shown in Chart 1, the reaction time of bilirubin with hydrogen peroxide was prolonged from twenty minutes to fifty-five minutes by reducing the amount of hydrochloric acid in the oxidizing solution from the usual 2 per cent to 1 per cent. With the use of a single monochromatic standard, however, the maximum blue color only is considered, and therefore, the more rapid reaction obtained with higher acid content is the more desirable.

Pure bilirubin may be used as a standard by treating the stock bilirubin solution in the same way as the unknown, but when it is used the solution in the colorimeter cup must be changed with each unknown, as the prolonged catalytic effect of the strong colorimeter light upon the oxidation of the standard

is sufficient to alter the results. Because of this difficulty the procedure is greatly facilitated by using a stable blue dye standard which is not affected by the light. Although the artificial standard is photostabile the oxidation of the pigment in the bile solution is hastened by the influence of strong light, and for this reason the reading of the unknown should be made promptly, once the solution is placed in the colorimeter. A standard composed of an alcoholic solution of an organic blue dye, nile blue sulphate, was found to be preferable to an aqueous solution of the inorganic salt, copper sulphate, which was previously used for this purpose.

#### SUMMARY

A simplified, rapid, and accurate method for the quantitative estimation of bilirubin in bile is presented in which hydrogen peroxide with its more constant and available source of free oxygen has been substituted for the nitric acid in the oxidizing reagent, thus eliminating errors due to the variability of the different specimens of nitric acid. The final colorimetric readings can be made within thirty minutes instead of the usual eighteen to twenty-four hours and at the maximum blue intensity instead of during the variable blue green color stage, making it possible to use a stable monochromatic organic dye, nile blue, as a standard for quantitatively estimating bile pigment.

The authors take great pleasure in expressing their sincere appreciation to Icie G. Macy, Ph.D., for her generous aid during the course of this work.

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## A METASTATIC TUMOR OF THE TRICUSPID VALVE ARISING IN A CASE OF MALIGNANT TERATOMA OF THE TESTIS\*

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THIS report deals with a case of teratoma of the testis in which an unusual tumor growth of the tricuspid valve of the heart was found at autopsy. The mass had produced no symptoms during life, and its presence was not suspected.

The history was briefly as follows: Seventeen months before death, the patient first noticed a swelling in the left side of the scrotum. This tumor grew rapidly, and on removal four months later was the size of an orange. The pathologic report on the specimen was teratomatous tumor of the testis.

Three months later he was readmitted to the hospital with a large mass in the left side of his abdomen. A laparotomy was performed and a large inoperable tumor the size of a grapefruit was found. Sections taken for biopsy were diagnosed secondary teratoma of the abdominal lymph glands. Microscopic sections showed the tumor to consist of a hemorrhagic and necrotic fibrous stroma containing early cartilage cells, cysts, and glands. The lining of the glands was of a low columnar epithelium, while some cysts were lined by a low cuboidal epithelium and others by stratified squamous epithelium.

Following discharge from the hospital, the patient's condition became rapidly worse, and on his last admission three months later, he complained of shortness of breath, pain in the right chest, weakness, and loss of weight. Physical examination revealed almost complete loss of resonance over the right chest, tracheal displacement to the left, clubbing of fingers and toes, and a large abdominal tumor.

At autopsy large tumor masses were found in the abdominal and pleural cavities. These tissues were irregular, soft, and hemorrhagic with many cystic areas scattered throughout the fibrous stroma. The microscopic picture was as above described.

Attached to the tricuspid valve were a number of curious, irregular masses made up of about 20 cystic structures (see Fig. 1). This tumor growth was 6 cm. in length and was firmly attached to the valve cusps, and with each of its cystic structures pedunculated and waving in the blood stream. Most of the tumor consisted of small yellowish white, firm, thick-walled cysts about the size of a match head joined together by fibrous connective tissue. There were also two large thin-walled cysts situated at either end of the mass. These cysts were soft, fluctuant, filled with a clear yellowish, watery fluid and measured 1.3 cm. in diameter.

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The principal tissue of the tumor was composed of a pale staining dense fibrous connective tissue. In some areas it was very compact and acellular, while in others, the wavy fibers were rich in deep staining nuclei, tending to be fusiform in shape.

The tumor masses were covered by a single layer of endothelial cells similar in appearance to those of the normal endocardium. Small thrombus masses



Fig. 1.—Gross appearance of cystic tumor of tricuspid valve.

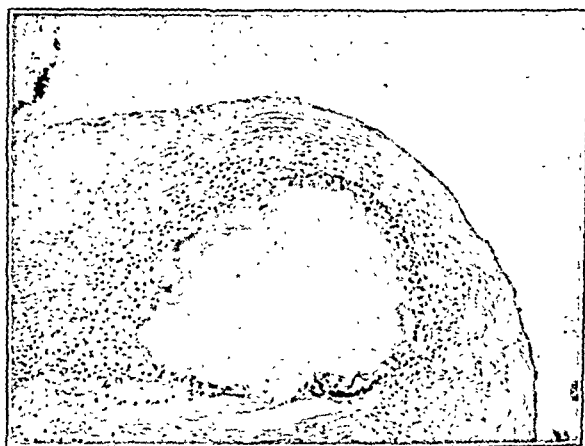


Fig. 2.—Microscopic section of epithelial lined cyst from tricuspid valve.

were found attached to the surface of the tumor. These consisted chiefly of a loose fibrin meshwork, containing many platelets and leucocytes. In a few areas the endothelial covering of the tumor was broken, and the thrombus was adherent to the fibrous tissue. The tissues were quite vascular.

The sections of an individual mass revealed several small cystic spaces. The lining of these cysts was variable in nature. One was lined in part by a

single layer of low cuboidal epithelial cells and partially by what appeared to be squamous epithelium. The cavity was filled by a small mass of desquamated epithelium. The fibrous connective tissue about the cyst was more dense and cellular than in other areas. One cystic cavity was completely lined by stratified squamous cells. This lining varied in thickness from five to fifteen cells, the upper layers being only slightly flattened. The basement membrane of the epithelium was very poorly defined. Occasionally the epithelium was of columnar character. There were also a few small clumps of cells scattered throughout the sections which presented the picture of early mesenchymal cells, showing a tendency to differentiate into cartilage.

#### SUMMARY

A tumor metastasis upon tricuspid valve is reported, the primary tumor being a malignant teratoma of the testis. The sections examined presented (1) a somewhat acellular fibrous connective tissue stroma, containing large areas of mucoid degeneration covered by a single layer of endothelium upon which were many thrombi; (2) the tissue contained cysts lined by both cuboidal and stratified types of epithelial cells; (3) there were present small capillary spaces filled by red blood cells; (4) small areas of mesenchymal cells suggestive of early cartilage formation were also present.

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### THE CAUSATIVE ORGANISMS OF PNEUMONIA IN CHILDREN IN EGYPT\*

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THIS work has been undertaken to find out the common causative organisms of pneumonia in children in Egypt for which purpose specimens taken directly by lung puncture at that part which clinically showed most physical signs were sent to the laboratory. The investigation was carried out during the three months from October to December, when pneumonias are most common in Egypt, particularly in the middle part of that period.

Fifty-two cases were available. Thirty-seven were out-patients (25 males and 12 females) and fifteen in-patients (10 males and 5 females). The ages varied from four months to five years.

Thirty-five patients exhibited lobular distribution (8 right, 11 left, and 16 bilateral). Ten were of the lobar type and 6 probably so (10 right and 6 left); one case was of the pseudolobar type (right). Of the former, one was associated with an empyema (syn-pneumonic), two with slight pleural effusion, and one with meningitis probably secondary to the lung condition. Of the lobar cases 3 were complicated with a syn-pneumonic empyema. The disease was primary in 31 cases, definitely secondary to whooping cough in 2, and prob-

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ably so in more, and secondary to infections of the upper respiratory tract in 14. In one patient the disease was of a tuberculous origin as verified by postmortem examination, although during life no evidence of tuberculosis was present.

The majority of out-patient cases could not be traced satisfactorily; two of the patients are known to have been cured. Of the in-patients 12 were cured, one improved, and 3 (one of them tuberculous) died.

#### EXPERIMENTAL

The specimens obtained by lung puncture and sent to the laboratory in sterile test tubes were plated out without delay on plates of blood agar and Fildes' peptic blood digest medium, incubated at 37° C. and examined after twenty-four hours and thereafter. To the remaining fluid in the test tubes broth was added, taking the necessary precautions to prevent contamination, and the tubes were likewise incubated at 37° C., so that in case the plated specimens proved sterile, there would still be hope of finding organisms by resorting to the broth cultures. These broth cultures, however, were only resorted to in very few instances, as it was possible in the great majority to obtain growth by primary cultivation. The colonies on these plates were examined, and pure cultures were made. Whenever necessary, biochemical and serologic reactions and pathogenicity tests were performed in order to determine the exact nature of the organism. With such organisms as frankly hemolytic streptococci and staphylococci, obviously such procedure was unnecessary, the appearance of the colonies and the films being quite sufficient. For the differentiation between Pfeiffer's bacillus and Bordet-Gengou's bacillus, the culture characteristics were largely depended upon, the better growth of the former on Fildes' medium and of the latter on Bordet's medium, etc. Sera were not available. Without producing all the protocols, it will be enough to mention that out of the 52 specimens sent for bacteriologic examination, 31 specimens were found to contain one kind of organism, 17 more than one, and 4 were sterile. The results are shown in Tables I and II.

TABLE I

CASES SHOWING THE PRESENCE OF ONE KIND OF ORGANISM. 31 SPECIMENS IN ALL

| NO. OF SPECIMENS | NATURE OF ORGANISM       | PER CENT |
|------------------|--------------------------|----------|
| 8                | Pneumococcus             | 16       |
| 5                | Hemolytic streptococcus  | 10       |
| 7                | Nonhemolytic             | 14       |
| 2                | Staphylococcus           | 4        |
| 5                | Pfeiffer's bacillus      | 10       |
| 4                | Bordet-Gengou's bacillus | 8        |

It will be seen that out of 52 cases, pneumococci were present in 13, hemolytic streptococci in 9, and nonhemolytic streptococci in 11 cases, either alone or in association with other organisms, and that these are evidently responsible for the great majority of cases.

As typing of pneumococci from cases of pneumonias was to our knowledge not done in Egypt, owing to the relative infrequency of pneumonias in adults and the difficulty of obtaining a reasonable number of strains to work with, it

TABLE II  
CASES SHOWING THE PRESENCE OF MORE THAN ONE KIND OF ORGANISM  
17 SPECIMENS IN ALL

| NO. OF SPECIMENS | NATURE OF ORGANISMS                                                                                          | PER CENT |
|------------------|--------------------------------------------------------------------------------------------------------------|----------|
| 2                | Pneumococcus and staphylococcus                                                                              | 4        |
| 1                | Pneumococcus and <i>N. catarrhalis</i>                                                                       | 2        |
| 1                | Pneumococcus and Friedlander's bacillus                                                                      | 2        |
| 1                | Pneumococcus and <i>M. tetragenus</i>                                                                        | 2        |
| 2                | Hemolytic streptococcus and staphylococcus                                                                   | 4        |
| 1                | Hemolytic streptococcus and diphtheroids                                                                     | 2        |
| 2                | Nonhemolytic streptococcus and staphylococcus                                                                | 4        |
| 2                | Nonhemolytic streptococcus and <i>B. Pfeiffer</i>                                                            | 4        |
| 1                | Staphylococcus and <i>B. Pfeiffer</i>                                                                        | 2        |
| 1                | Staphylococcus and <i>N. catarrhalis</i>                                                                     | 2        |
| 1                | Staphylococcus and Bordet-Gengou's bacillus                                                                  | 2        |
| 1                | Staphylococcus and Friedlander's bacillus                                                                    | 2        |
| 1                | <i>B. Pfeiffer</i> , hemolytic streptococcus and an aerobic terminal spore bearer (probably a contamination) | 2        |

TABLE III

| TYPE OF DISEASE              | CAUSATIVE ORGANISM                                             | NO. OF CASES |
|------------------------------|----------------------------------------------------------------|--------------|
| Definitely lobar<br>11 cases | Pneumococcus                                                   | 4            |
|                              | Pneumococcus and <i>B. Friedlander</i>                         | 1            |
|                              | Hemolytic streptococcus                                        | 2            |
|                              | Hemolytic streptococcus and diphtheroids                       | 1            |
|                              | Pneumococcus and <i>M. tetragenus</i>                          | 1            |
|                              | Staphylococcus and <i>N. catarrhalis</i>                       | 1            |
|                              | <i>H. influenzae</i>                                           | 1            |
| ? Lobar<br>6 cases           | Hemolytic streptococcus and staphylococcus                     | 1            |
|                              | <i>B. influenzae</i>                                           | 3            |
|                              | Sterile                                                        | 1            |
|                              | Bordet-Gengou's bacillus                                       | 1            |
| Lobular<br>35 cases          | Sterile                                                        | 3            |
|                              | Pneumococcus                                                   | 4            |
|                              | Hemolytic streptococcus, <i>H. influenzae</i> and spore bearer | 1            |
|                              | Pneumococcus and <i>N. catarrhalis</i>                         | 1            |
|                              | Pneumococcus and staphylococcus                                | 2            |
|                              | Hemolytic streptococcus                                        | 3            |
|                              | Bordet Gengou's bacillus                                       | 3            |
|                              | Nonhemolytic streptococcus                                     | 7            |
|                              | Nonhemolytic streptococcus and staphylococcus                  | 2            |
|                              | Staphylococcus                                                 | 2            |
|                              | Staphylococcus and <i>Hemophilus influenzae</i>                | 1            |
|                              | <i>H. influenzae</i>                                           | 1            |
|                              | Nonhemolytic streptococcus and <i>H. influenzae</i>            | 2            |
|                              | Hemolytic streptococcus and staphylococcus                     | 1            |
|                              | Staphylococcus and Bordet-Gengou's bacillus                    | 1            |
|                              | Staphylococcus and <i>B. Friedlander</i>                       | 1            |

was thought advisable to do typing on the few available strains of pneumococci isolated from our cases. For typing, sera sent us by courtesy of the Rockefeller Institute were used and for the preparation of emulsions pure cultures on serum agar were employed. The serial method of dilution of serum was adopted, and the mixtures incubated in a water-bath at 50° C. for four hours. The result is shown in Table IV.

TABLE IV

| TYPES OF PNEUMOCOCCUS | NO. OF SPECIMENS | PER CENT |
|-----------------------|------------------|----------|
| Type I                | 2 cases          | 15       |
| Type II               | 3 cases          | 24       |
| Type III              | 2 cases          | 15       |
| Group IV              | 6 cases          | 46       |

It must be admitted that the number of strains is hardly sufficient to come to any conclusion as to the distribution of types of pneumococci isolated from cases of pneumonia in Egypt. It is our intention to proceed with the collection of more strains in order to be able to give more reliable results, but the figures as they are will give an approximate idea about the distribution of the various types.

#### SUMMARY AND CONCLUSIONS

1. Fifty-two cases of pneumonias occurring in infancy and childhood in Egypt were examined, 35 being of the lobular and 17 of the lobar type.

2. The disease was primary as far as the history and examination showed in 3 cases and secondary in the remainder, being secondary apparently to whooping cough in 6, and to infections of the upper respiratory tract in 14.

3. In 31 instances a single kind of organism was found by lung puncture (59.6 per cent); more than one kind of organism were found in 17 cases (32.7 per cent), and the puncture was sterile (7.5 per cent) in 4 instances.

4. Altogether pneumococci were present in 13 cases (25 per cent) and streptococci in 20 cases (38.5 per cent) (hemolytic in 9 and nonhemolytic in 11), either alone or in association with other organisms. These organisms seem to be responsible for most of the cases examined.

5. Of the cases showing one kind of organism, pneumococci were found in 8, streptococci in 12 (hemolytic in 5, nonhemolytic in 7), staphylococci in 2, Pfeiffer's bacillus in 5, and Bordet-Gengou's bacillus in 4 cases.

6. Typing of the pneumococci met with showed 15 per cent of Type I, 24 per cent of Type II, 15 per cent of Type III, and 46 per cent of Group IV, but the limited number of cases prevents us from considering these percentages as final.

We wish to express our gratitude to Dr. I. Shawky and Dr. A. Khalil of the Pediatric Department, Faculty of Medicine, Cairo, for their valuable suggestions.

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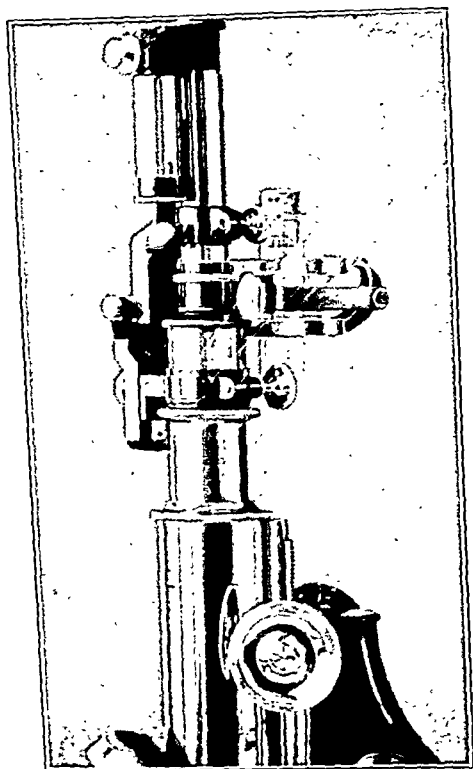
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# LABORATORY METHODS

## A NEW MICROSCOPE ADAPTER FOR THE HAND SPECTROSCOPE\*

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IN A recent communication I† described a method for the routine examination of blood stains in medicolegal cases. The method requires spectroscopic examination of suitably treated material on a microscopic slide, and this involves the insertion of the body of a hand spectroscope into the tube of the microscope.



I found that it was not easy to hold the spectroscope steady and at the same time make an exact reading of the wave length scale. I discussed the difficulty with the representative of Messrs. Zeiss & Co. and described to him more or less what was wanted, and the attachment presently described is the result of

\*From the Bacteriological Institute, Medical Department.

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†Greaves, A. V.: The Use of Takeyama's Solution in the Identification of Blood Stains, Brit. M. J. 2: 932, 1932.

the leaven of my suggestion working on the dough of their ingenuity. Reference to the figure will show that the body of the spectroscope is grasped by a circular band tightened by a screw with a milled head. This is connected with a heavily built standard, which in turn, is connected by a similar band attachment to the draw tube of the microscope. The spectroscope is thus held rigidly in perfect alignment with the optical system of the microscope. The standard is broken in the middle by a hinge by means of which the spectroscope may be swung away from the observer and thus free use of the ocular permitted. In the illustration may also be seen an attachment with reflecting mirror for use with a comparison prism if the spectroscope is so equipped. This may be removed if desired. The smaller tube containing the wave length scale is also equipped with a small reflecting mirror by means of which a beam of light may be passed up the tube to illuminate the scale. In the photograph the microscopic ocular is shown in place; I have found that it is much better to remove it before observing the spectrum. The method suggested for use of the appliance is as follows: The attachment (which may conveniently be left permanently applied to the spectroscope) is slipped over the tube of the microscope (after removal of the ocular) and the screw on the band tightened, so that the end of the spectroscope tube is just clear of the tube of the microscope. The hinge of the holder is adjusted so as to be on the side of the tube away from the observer. The microscope is now placed accurately in relation to the lighting system and the small mirror at the end of the tube bearing the wave length scale is adjusted so as to illuminate the scale. When these preliminary preparations have been made, the spectroscope may be swung away from the observer and the ocular replaced in the tube and the microscope is then ready for use as soon as the slide preparation is made. When the color change indicative of the presence of blood is observed on the slide (after treatment with Takayama's solution), the ocular is withdrawn, the spectroscope is swung into place and the spectrum observed and the bands located in relation to the scale. The appliance is heavily built, and there appears to be nothing about it which can get out of order or become worn.



## A CENTRIFUGE METHOD FOR THE DETERMINATION OF THE VOLUME OF CELLS IN BLOOD\*

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IN RECENT years increasing attention has been paid to the fact that significant changes of the volume of cells in the blood may be observed in a wide variety of pathologic conditions, and that in certain diseases the determination of these changes may afford valuable diagnostic aid. Knowledge of the relative volume of cells, of the red cell count and of the concentration of hemoglobin in a sample of whole blood permits the calculation of two values which are increasingly used in the classification of different types of anemia; namely, the average size of the erythrocytes in cubic microns and the concentration of hemoglobin per unit of volume in the red cells. Reviews of most of the reported studies of this subject may be found in articles by Haden (1931, 1932), Osgood, Haskins, and Trotman (1932), and Wintrobe (1932). The determination of the relative volume of cells in blood also permits the indirect estimation of various chemical constituents of the cells by calculation from determinations made on whole blood and plasma in circumstances where direct chemical study of the cells is less satisfactory.

Numerous methods have been offered for the determination of blood cell volume, but most of them have certain features which present more or less serious obstacles to their general application. Ponder and Saslow (1930) have reviewed many of the methods and discussed the faults inherent in each type of procedure. These authors claimed that their modification of the Stewart (1899) colorimetric method was the most accurate means of determining cell volume, but this method is extremely tedious and the amount of blood required is prohibitive if the bloods of infants or small laboratory animals are to be studied. Although the centrifuge method in its many modifications has been criticized by Ponder and Saslow as well as by others who have studied the problem, this procedure is, nevertheless, generally considered to be the most practical for clinical use. Aside from other advantages, the hematocrit tube has one distinctive point of superiority over other devices, in that it offers the only practical means of measuring separately the total cell volume and the red cell volume. This is important if the cell volume value is used to calculate the size of the erythrocytes or the hemoglobin content of the red cells, since the failure to exclude the large white cells may often introduce into these calculations an easily perceptible error.

Judging by the published reports of cell volume studies, the Van Allen (1925) method appears to be one of the most popular of the several procedures in current clinical use. The Van Allen tube is convenient and is economical of

\*From the Children's Hospital Research Foundation and the Department of Pediatrics, College of Medicine, University of Cincinnati.  
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blood, but it has one potential source of error in common with all types of hematocrit tubes with which rubber sealing devices are used; such sealing devices occasionally allow slight leakage which may pass unnoticed, and this danger is increased the higher the centrifuge speed to which the tubes are subjected. Another objection to this method is that the diluting fluid (1.3 per cent sodium oxalate solution) while perhaps isotonic for normal blood, may not necessarily be isotonic for pathologic blood samples in which osmotic changes have occurred. This criticism is applicable to all those methods employing anticoagulant salts which may cause changes of cell volume. Whether a supposedly isotonic oxalate solution is used as in the Van Allen (1925) and Haden (1923, 1925) methods, or whether dry oxalate is used and correction made for shrinkage of the cells, as in the Osgood (1926) and Wintrobe (1929) methods; in either case the constants determined for normal bloods have not been proved dependable for bloods in which osmotic changes have occurred under different pathologic conditions.

Economy of blood is nearly always an important consideration and at times is essential in these studies. Haden (1930) maintained that 10 c.c. of blood—the amount used in his method—was not an excessive amount to take from a patient. This is perhaps true of adults, but it is usually impractical to remove such an amount from infants, especially when more blood is to be drawn for other chemical studies. In discussing various methods Haden also claimed that a macromethod was necessary for accuracy, but in our own experience it has been possible to make closer measurements in capillary tubes than in larger tubes. It is, moreover, easier to differentiate the layer of white cells if the columns of cells are measured in capillary tubes.

Gram and Norgaard (1923) employed a capillary tube with heavy walls, 10 cm. in length and graduated in 200 intervals, with hirudin as anticoagulant. Later Rosahn (1931) devised a tube somewhat similar to that of Gram and Norgaard and used heparin as the anticoagulant. In both procedures the tubes were sealed with rubber bands for centrifuging.

In several methods employing capillary tubes the use of rubber sealing devices has been avoided. Smirk (1929) used thin-walled capillary tubes of 0.3 mm. bore, bent into U-shape. Oxalated blood was drawn into such tubes with a layer of paraffin oil at each end of the column of blood to diminish the loss of  $\text{CO}_2$ , and the tubes were centrifuged at 3,000 to 4,000 r.p.m. until the cells were packed to constant volume. The lengths of the columns of cells and plasma were then marked on paper by pricking points, the distances between the points measured with dividers, and the cell volume in percentage calculated from these measurements. The results from the two columns of the U-tube were averaged. Smirk claimed that, at the same speed of rotation, more complete separation of plasma from cells was obtained by centrifugation in capillary tubes than in larger tubes, and that separation was more complete in shorter columns of blood, 5 to 6 cm., than in longer columns. Bönniger (1909) also had employed a U-shaped tube. Campbell (1922) used thin-walled capillary tubes which were partly filled with oxalated blood by dipping one end into the blood, and the opposite end then sealed in a flame. After centrifugation the lengths of the columns of cells and plasma were measured by a millimeter scale and the

percentage cell volume calculated. Epstein (1915) used heavier capillary tubes, of 0.5 to 1.0 mm. bore, with one end drawn out to be sealed in a flame. He placed granules of heparin in the tube and used blood obtained by skin puncture. After the tube was partly filled with blood and the small end sealed, the blood column was measured end to end and the thin end of the tube inserted into a cork for centrifugation. After centrifugation the length of the column of plasma was measured, the plasma volume was calculated in percentage and the volume of cells obtained by difference.

The method for the determination of the relative volume of cells in blood which is to be described here has been developed after a considerable amount of experimental work with many of the reported centrifuge methods; and various steps in the procedure have been adopted, with modifications, from a number of different sources. Briefly: in this method thin-walled capillary tubes in pairs are partly filled with heparinized blood, one end sealed in a flame, and the tubes thus filled are centrifuged until the cells are packed to constant volume. The columns of cells and plasma are then measured by means of a measuring microscope, and from these measurements the volume of "total" cells and "R.B.C." are calculated in percentage of whole blood. A high speed centrifuge with special head and a special measuring microscope are recommended, but practically identical results may be obtained with the ordinary types of laboratory centrifuge and microscope if the latter be equipped with a cross-hair eyepiece and a graduated mechanical stage. The principal advantages of the method are that it is economical of blood; that the anticoagulant used apparently does not affect the size of the cells; that the volumes of red cells and white cells may be read separately with ease; that errors due to rubber sealing devices are avoided; that it is rapid.

#### APPARATUS

Vials to receive the blood samples may be prepared, a large number at a time, by placing in small vials appropriate amounts of a 1 per cent solution of heparin\* in distilled water. Convenient sizes are 2 c.c. or 4 c.c. vials which receive respectively 0.1 or 0.2 c.c. of this solution; i.e., 1.0 or 2.0 mg. of heparin. The water is then evaporated from the vials in a drying oven at 100° C., or in a vacuum oven at a lower temperature. In either case the vials should not be left in the oven longer than necessary, because after prolonged heating the heparin tends to dissolve in the blood less readily. When dry, a glass bead and a closely fitting rubber stopper are placed in each vial.

*Capillary tubes* are prepared from ordinary soft glass tubing (internal diameter 10 mm. and wall 1.0 mm. in thickness) which has been cleansed with acid cleaning mixture, rinsed with distilled water and dried. This tubing is heated over a Bunsen burner with wing top adjusted to give as even a flame as possible, and the heated portion is drawn out 15 or 20 feet by two people walking rapidly away from each other. The pulled capillary tube should be held taut until well cooled, and then dropped onto a flat surface in order to

\*The heparin is obtainable from Hynson, Westcott and Dunning, Baltimore. Several writers have suggested the advantages of the use of heparin in cell volume studies, but dismissed it as too expensive. The use of small blood samples reduces the cost, and in this method of drying there is little waste.

minimize its tendency to warp into curves. From this capillary tubing 12.0 cm. lengths are cut and carefully selected for straightness and absence of taper. Such selection may be made conveniently by rolling the capillaries on a flat surface (plate glass) which is slanted a few degrees. The curved tubes are culled out by their failure to roll, and a significant degree of taper will cause the tubes to roll to one side. The most suitable capillary tubes may range in internal diameter from 0.4 to 0.8 mm., with walls 0.1 to 0.15 in thickness. In our experience the yield of acceptable tubes prepared in this fashion is from 20 to 40 per cent. Because the heat employed in drawing the tubing may cause some alkali to appear on the surface of the glass, the selected capillaries should be dropped into acid, rinsed with water and dried. Such rinsing is easily done by placing a number of the capillary tubes in a glass cylinder or test tube which is filled and emptied several times in succession with acid, distilled water, alcohol, and ether. After the final washing with ether, the capillaries may be placed in a drying oven for a short time to drive out the ether.

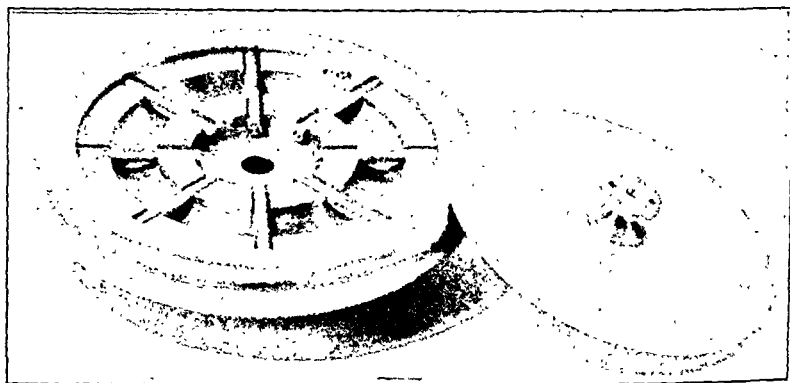


Fig. 1.—Centrifuge head used with the high speed attachment of the International centrifuge.

The labor involved in preparing the capillary tubes is somewhat compensated by the fact that the tubes when used once are thrown away, and no time is spent cleaning them.

*Centrifuge.*—The special head shown in Fig. 1 may be used for centrifuging the capillary tubes on the high speed attachment of the International centrifuge.\* This head has a radius of 75 mm., and has places for eight small metal cylinders, each bored to carry a pair of 6 cm. capillary glass tubes. The tubes are carried horizontally, in their plane of motion, so that at the end of centrifuging the tops of the columns of packed cells are level. With this head the best results have been obtained by a speed of 14,000 r.p.m. for from four to five minutes. An automatic timing device to stop the centrifuge is helpful.

For centrifuging in the usual style cups with an ordinary type of centrifuge capable of speed around 3,000 r.p.m., carriers for the capillary tubes can be made from a block of wood or aluminum made to fit loosely in a centrifuge cup and bored with small holes, each hole just large enough to carry a pair of the capillary tubes. Several pairs can be carried in one such block.

\*The International Equipment Co., Boston, Mass.

*Measuring Microscopes.*—In all the control studies listed here the measurements of the columns of blood cells and plasma after centrifuging were made by means of the measuring microscope shown in Fig. 2.\* The positions of the microscope, the light source, etc., as shown in the figure, are arranged for the comfort of the operator. Two pieces of opaque paper are pasted on a plate of glass so as to make a horizontal narrow slit in which the capillary tube is placed for the measurements. The light is reflected from a mirror through the slit, and readings are made by transmitted light which makes the line of demarcation between white and red cells more distinct. While this microscope permits readings to 0.01 mm., any laboratory microscope (low power objective) equipped with a cross-hair eyepiece and a graduated mechanical stage with vernier which permits readings to 0.1 mm. gives results practically identical

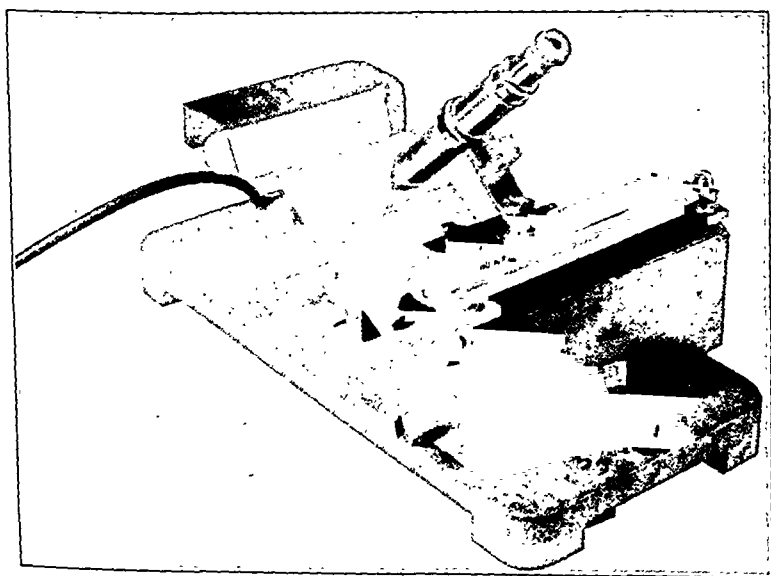


Fig. 2.—The measuring microscope, arranged for measuring the lengths of columns of blood cells and plasma.

with those obtained by closer readings. For use with the ordinary microscope, a holder for the capillary tubes may be made by pasting two pieces of opaque paper on a glass slide so as to make a narrow slit in which the capillary tube is placed during its measurement. Light is reflected through this slit from below by the mirror of the microscope substage.

#### METHODS

The blood sample for the cell volume determination should be obtained with a dry syringe and needle and quickly mixed with the heparin, 2 c.c. of blood to 1.0 mg. of the anticoagulant, in a small vial such as previously described. Care should be observed to avoid air bubbles in the blood discharged into the vial, and mixing of the anticoagulant should be by gentle inversion of

\*Obtained from the Central Scientific Company, Chicago, Illinois.

the tube without violent shaking which is likely to produce foaming. If tissue juices are mixed with the blood, or if slight clotting of the blood starts in the syringe before it is discharged into the heparin vial the formation of particles of fibrin may cause difficulty even though the blood appears grossly to be unclotted. With slight fibrin formation the line of demarcation between the red and white cells is harder to read and sometimes the white cells are found scattered through the red cells, apparently caught in the strands of fibrin. The heparinized blood may be used also for cell counts, hemoglobin estimation and any other chemical examinations with which heparin does not interfere.

At the time of use one of the 12.0 cm. capillary tubes is cut in the middle to give a pair of tubes which are turned to bring their "outer" ends together. Several pairs (usually four in our own work) should be used for each blood sample. The "outer" ends of each pair of tubes are thrust deeply into the *well mixed* blood and filled by capillarity to within about 2.0 cm. of the "inner ends"—i.e., the ends that came from the middle of the original tube. Finally a layer of paraffin oil 2.0 to 3.0 mm. deep may be drawn into the tubes over the columns of blood. The oil serves mainly to prevent evaporation of water from the surface of the plasma; it is doubtful whether it diminishes the loss of  $\text{CO}_2$ , as claimed by Smirk who used it in a similar fashion. The oil is not essential to secure results sufficiently accurate for routine clinical work. The "inner" ends are then sealed by holding them perpendicular to and just touching the outer margin of a micro burner gas flame. With care and slow rotation the tube can be sealed quickly with a flat inner base. It is important that this be done carefully, since slight taper in the sealed base of the tube makes the final measurement of the lengths of the blood column less accurate. If the wall of the tube is too thick, or if the glass has not a low melting point, it is difficult to make such a seal quickly and the glass may become hot so far up the tube that there is danger of overheating the lower part of the blood column. This manner of sealing excludes the possibility of even slight loss of blood by leakage which is a frequent source of error in hematocrit tubes with rubber sealing devices. The advantage of having the pair of tubes cut from one longer tube, and turned as described, lies in the fact that the slight taper usually present even in the most carefully selected capillaries is then in the opposite direction in each tube of the pair, and, therefore, the error due to taper in the one compensates that of the other in the final results.

The tubes thus filled and sealed are centrifuged, either in the special head (Fig. 1) at approximately 14,000 r.p.m. for from four to five minutes, or in the ordinary centrifuge at approximately 3,000 for thirty minutes. In samples of dog's blood with red cell counts as high as eight million or more, the period of centrifuging at 14,000 r.p.m. should be extended to five or six minutes. With slower speeds a longer time is necessary to completely pack the cells; with speeds higher than 14,000 r.p.m. the red cells in some blood samples tend to rupture and the hemoglobin becomes diffused into the layer of white cells, making the line of demarcation between the red and white cells harder to read. After centrifuging, the capillary tubes are immediately placed upright in a block of wood bored with small holes to receive them; if left lying horizontally for more than a few minutes the top of the layer of cells will be slanted.

For the measurements, each capillary tube is placed in the light slit of the glass slide before the measuring microscope. With the stage of the latter set at zero, the capillary tube is adjusted by hand to bring the base of the blood column to the cross-hair of the eyepiece; the stage is then moved laterally and readings are made at the line of demarcation between the red and white cells, at the top of the white cells and at the meniscus of the plasma. From these measurements on each tube the cell volumes, "R.B.C." and "Total," are calculated in percentage of whole blood. The values for each pair of tubes are averaged (as shown in Table I), and as a rule the maximum variations between these averages in a set of four pairs of tubes is less than 0.4 per cent.

#### CONTROL STUDIES

*Internal Diameter of the Capillary Tubes.*—Two sets of capillary tubes were selected, the capillaries in each set having internal diameters 0.3 to 0.4 mm. and 0.6 to 0.8 mm., respectively. Six pairs in each set were filled alike, sealed in a flame as described and centrifuged at 12,000 r.p.m. for four minutes. The averages for the total cell volumes calculated for the two sets of tubes were respectively 45.41 and 45.39 per cent. Although there was found no significant difference between the results from the two sets, practically the tubes with internal diameters from 0.5 to 0.7 mm. are the most satisfactory to use. The smaller ones are too fragile; while the larger ones are harder to seal with a good flat inner base, and the thicker blood column makes the line of demarcation between the white and red cells harder to read by transmitted light.

*The Effect of Taper in the Capillary Tubes (Table I).*—It was previously stated that the reason for having the pair of capillary tubes cut from one longer tube is that they may be turned so that if slight taper is present, it will be in the opposite direction in each tube of the pair and the error due to taper in one will compensate that of the other. This effect is illustrated in Table I by the readings of a set of tubes in which the differences are considerable between the members of each pair and yet the agreement is good between the averages of the separate pairs.

TABLE I  
EFFECT OF TAPER IN THE CAPILLARY TUBES

| READINGS IN MILLIMETERS |        |        | CALCULATED CELL VOLUMES IN PER CENT |                    |       |                    |
|-------------------------|--------|--------|-------------------------------------|--------------------|-------|--------------------|
| R.B.C.                  | W.B.C. | PLASMA | R.B.C.                              | AVERAGE<br>OF PAIR | TOTAL | AVERAGE<br>OF PAIR |
| 23.60                   | 24.13  | 50.80  | 46.46                               |                    | 47.50 |                    |
| 24.06                   | 24.56  | 51.04  | 47.14                               | 46.80              | 48.12 | 47.81              |
| 23.11                   | 23.58  | 50.97  | 45.34                               |                    | 46.26 |                    |
| 25.43                   | 25.97  | 52.96  | 48.02                               | 46.68              | 49.04 | 47.65              |
| 23.65                   | 24.13  | 51.22  | 46.17                               |                    | 47.11 |                    |
| 24.50                   | 24.99  | 51.85  | 47.25                               | 46.71              | 48.20 | 47.66              |
| 24.53                   | 25.16  | 52.10  | 46.81                               |                    | 48.02 |                    |
| 23.99                   | 24.59  | 51.41  | 46.66                               | 46.74              | 47.83 | 47.93              |
| General Average         |        |        |                                     | 46.7               |       | 47.8               |

*Length of the Columns of Blood (Table II).*—Pairs of capillary tubes, 6 cm. in length and from 0.4 to 0.6 mm. in internal diameter, were filled with heparinized blood sufficiently to give blood columns ranging from 20 to 55 mm. in total length. These were sealed in a flame as usual and centrifuged at 12,000 r.p.m. for four and one-half minutes. The results listed in Table II indicate that the length of the columns of blood within these limits affects the final results very little.

TABLE II  
EFFECT OF LENGTH OF BLOOD COLUMNS

| LENGTH OF<br>BLOOD COLUMNS<br>MM. | NUMBER OF<br>PAIRS | CELL VOLUMES IN PER CENT |                |
|-----------------------------------|--------------------|--------------------------|----------------|
|                                   |                    | R.B.C.                   | TOTAL          |
| 20-29.9                           | 7                  | 45.63                    | 46.53          |
| 30-39.9                           | 7                  | 45.74                    | 46.53          |
| 40-55.0                           | 9                  | 45.72                    | 46.45          |
| Total Pairs                       | 23                 |                          |                |
| Mean                              |                    | 45.70 ± 0.03             | 46.51 ± 0.02   |
| Standard Deviation                |                    | ± 0.18 ± 0.02            | ± 0.16 ± 0.02  |
| Coefficient of Variation          |                    | ± 0.38% ± 0.04           | ± 0.35% ± 0.03 |

*Time of Centrifuging at 14,000 r.p.m. Within a 7.5 cm. Radius (Table III).*—From a sample of heparinized normal human blood, 7 sets of capillary tubes, 4 pairs in each set, were filled and sealed as described. These sets in turn were centrifuged in the special head at 14,000 r.p.m. for periods from one-half minute to ten minutes. During the one-half minute period high speed was not attained. The results shown in Table III indicate that at 14,000 r.p.m., four to five minutes is sufficient to obtain constant packing of the cells in human blood. In this centrifuge, run at this speed, periods longer than ten minutes were undesirable because if run a longer time the apparatus tended to become heated.

It may be noted here that in the capillary tubes translucency of the blood cell column (Koeppé's criterion [1905]) cannot be accepted by itself as a criterion of complete packing of the cells; a fair degree of translucency sometimes may be observed before the cells are completely reduced to constant volume. The conditions of speed and time must be adjusted to obtain this maximum packing of the cells (without rupture of the cells), but on the other hand too long periods of centrifuging should be avoided because of chemical changes which may occur in the cells and alter their volume.

TABLE III  
TIME OF CENTRIFUGING AT 14,000 R.P.M.

| TIME<br>MINUTES | CELL VOLUMES IN PER CENT |       |
|-----------------|--------------------------|-------|
|                 | R.B.C.                   | TOTAL |
| ½               | 61.88                    | 62.75 |
| 1               | 48.15                    | 49.01 |
| 2               | 46.85                    | 47.59 |
| 3               | 46.50                    | 47.27 |
| 4               | 46.45                    | 47.31 |
| 6               | 46.44                    | 47.26 |
| 10              | 46.51                    | 47.25 |



*Time of Centrifuging at 3,000 r.p.m., Within a 20 cm. Radius.*—From a sample of heparinized normal human blood 5 sets of capillary tubes, 4 pairs in each set, were filled and sealed as described. Four sets were centrifuged in a 50 c.c. cup and holder, such as described under apparatus, with a No. 1 International centrifuge (Type SB) using a head with 15 cm. radius (the bottom of the cup traveled at a 20 cm. radius). When these 4 sets were centrifuged at 3,000 r.p.m. for fifteen, thirty, forty-five, and sixty minutes, the average total cell volumes measured in these sets were respectively 44.4, 44.2, 44.0, and 44.2 per cent. In the fifth set, centrifuged in the special head at 14,000 r.p.m. for four minutes, the total cell volume was 44.1 per cent.

In ordinary practice, therefore, centrifuging the capillary tubes to constant cell volume in the ordinary laboratory centrifuge should usually give results as satisfactory as those obtained with the special high speed centrifuge. In certain pathologic conditions, however, there is need to handle blood samples as quickly as possible after the blood is drawn, because rapidly progressing intracellular chemical changes may alter the size of the cells. For example, in certain types of severe acidosis and in gastroenteric intoxication of infants, it may be observed that glycolysis, lactic acid formation and liberation of inorganic phosphates by intracellular hydrolysis of organic phosphorus compounds occur more rapidly than in normal bloods (Guest [1932]). In circumstances such as these, the shorter period of centrifugation at higher speed is advantageous.

*Effect of Standing at Room Temperature (Table IV).*—Heparinized normal human blood was allowed to stand at room temperature (28° to 32° C.; this experiment being done in the summer) for seventeen hours. At intervals after the blood was drawn, indicated in Table IV, sets of four pairs of capillary tubes were filled, sealed, and centrifuged at 13,500 r.p.m. for four and one-half minutes. The results listed in Table IV indicate that in the first three hours there was a noteworthy shrinkage in the cell volume; part of this effect may be due to loss of CO<sub>2</sub> from the blood, and part to changes concomitant with glycolysis. In the later periods the increased amount of lactic acid accumulating as a result of glycolysis may be responsible for the swelling of the cells.

TABLE IV  
THE EFFECT OF STANDING, AT ROOM TEMPERATURE

| TIME AFTER BLOOD<br>WAS DRAWN | CELL VOLUMES IN PER CENT |       |
|-------------------------------|--------------------------|-------|
|                               | R.B.C.                   | TOTAL |
| 15 minutes                    | 45.8                     | 46.4  |
| 1½ hour                       | 45.4                     | 46.2  |
| 3 hours                       | 44.6                     | 45.4  |
| 6 hours                       | 45.1                     | 45.8  |
| 11 hours                      | 46.2                     | 47.0  |
| 17 hours                      | 46.8                     | 47.5  |

*Anticoagulants (Table V).*—A sample of normal human blood was drawn and delivered in 5 c.c. samples into small vials containing the following: (1) heparin 2.0 mg., (2) hirudin 2.0 mg., (3) sodium citrate 0.015 gm., (4) sodium oxa-

late 0.015 gm., (5) sodium fluoride 0.015 gm. Another sample, No. 6, was defibrinated with a glass rod. Four pairs of capillary tubes were prepared from each of these samples and centrifuged as usual. The results of the test are listed in Table V. It may be noted that identical results were obtained with the use of heparin and hirudin.

TABLE V  
EFFECT OF DIFFERENT ANTICOAGULANTS

| ANTICOAGULANT   |       | CELL VOLUMES IN PER CENT |       |
|-----------------|-------|--------------------------|-------|
|                 |       | R.B.C.                   | TOTAL |
| Heparin         | 0.04% | 45.1                     | 45.8  |
| Hirudin         | 0.04% | 45.1                     | 45.8  |
| Sodium citrate  | 0.3 % | 41.7                     | 42.4  |
| Sodium oxalate  | 0.3 % | 39.5                     | 40.0  |
| Sodium fluoride | 0.3 % | 36.3                     | 36.8  |
| Defibrinated    | -     | 44.8                     | 45.3  |

*Amount of Heparin.*—A sample of normal human blood was added to heparin in two vials in the proportion 0.5 mg. per c.c. and 2.0 mg. per c.c., respectively. Eight pairs of capillary tubes were filled from each vial and centrifuged at 14,000 r.p.m. for four minutes. The mean values for total cell volume in the two sets were respectively  $41.92 \pm 0.11$  and  $41.84 \pm 0.11$  per cent; two values with no significant difference by mathematical test. It may be assumed, therefore, that between these limits the proportion of heparin added to blood need not be accurately measured.

*Types of Glass Used in the Capillary Tubes.*—Because it is easier to draw capillary tubing from lead glass and to obtain a high yield of straight capillary tubes, it was hoped that this type of glass could be used. However, when sets of lead glass and soda lime glass capillary tubes were used for the same blood samples, the lead glass tubes gave results for cell volume about 2 volumes per cent lower than those obtained with the soda lime glass tubes. When these capillary tubes were kept for twenty-four hours after the determinations, it was observed that the packed cells in the lead glass capillary tubes become increasingly opaque, whereas, in the soda lime glass tubes the column of cells remained translucent. It seems likely that the lower cell volume was due to the effect of lead entering the blood from the glass. Pyrex glass is much more difficult to draw into capillary tubing sufficiently uniform for the needs of this method, and it is more difficult to seal Pyrex glass capillaries properly without overheating the blood in the tube.

*Comparison of Cell Volume Determinations by Other Methods.*—Twenty-five cubic centimeters of blood were drawn from a normal human adult and this sample divided for cell volume measurements according to four different methods: (1) The Sanford-Magath (1929) modification of the Haden method, (2) Van Allen method (1925), (3) Wintrobe (1929) method, (4) the capillary tube method as here described. Results averaged for several tubes used in each of these methods were respectively 45.4, 45.1, 47.9, and 47.3 per cent for the total cell volumes.

Studies of blood cell volume and erythrocyte size in arterial and venous blood, the effects of contact of the blood with air and changes in  $\text{CO}_2$  content,

and the effects of stasis of blood in veins before blood samples are drawn, will be reported in a later communication. It may be stated here, however, that when blood samples are handled as described in the foregoing pages, the slight losses of  $\text{CO}_2$  which may result from brief contact of the blood with air have practically no effect upon the cell volume.

## SUMMARY

A method for the determination of the percentage volume of cells in blood has been presented. Small capillary glass tubes in pairs are partly filled with heparinized blood, the tubes sealed in a flame, and the tubes then centrifuged until the cells are packed to constant volume, preferably at 14,000 r.p.m. for from four to five minutes in a special centrifuge head. The lengths of the columns of cells and of plasma are read either by a special measuring microscope or by means of an ordinary microscope equipped with a graduated mechanical stage. The readings are made to the top of the red cells, to the top of the white cells, and to the top of the plasma. From these measurements the relative cell volumes "R.B.C." and "Total" are calculated in percentage of whole blood. Control studies are presented to indicate the accuracy and variations to be expected in measurements made by this method.

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## A PRACTICAL METHOD FOR THE CONTINUOUS ADMINISTRATION OF FLUID INTRAVENOUSLY\*

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WHEN the intravenous administration of liquid is desirable, an efficient apparatus designed to perform this service is welcome. Such an apparatus must accomplish its function with safety and dependability, as well as provide for the comfort of the recipient, if it is to compete with other methods.

The apparatus here described was designed specifically for the intravenous administration of liquids at constant rates for long periods of time. Its primary purpose is to deliver liquid into the veins of sick patients under conditions determined by them, meanwhile satisfying the requirements of asepsis, of safety, of dependability, and of ease of manipulation.

Although it will probably find its greatest uses clinically in those cases requiring long treatment, this apparatus is also well adapted for use in cases where no cooperation is offered by the recipient. In the laboratory, when mounted on a rotating platform on top of a cage, it becomes an efficient instrument for the continuous intravenous injection of animals.

The apparatus, which is shown in the photograph (Fig. 1), consists of (1) an eccentric rotor driven by a variable-speed synchronous type motor, and (2) an independent tube for conducting the liquid. The pump itself operates on the well-known principle of "milking a tube," modified to avoid all connection between pumping mechanism and conducting system. A powerful propulsion of liquid is effected by the progression of the eccentric rotor over a spiral segment of the conducting tube. The rest of the apparatus embodies several other principles which guarantee the uninterrupted injection of sterile liquid even without the cooperation of the recipient.

In order to assure a smooth and uninterrupted one-way flow, the liquid is carried under pressure to the point of discharge, and is there released to flow freely into the vein. This operation is achieved through use of a release valve at the end of the conducting tube which not only offers resistance to the forward flow, but also prevents the backward flow of liquid. The valve consists of a small piece of rubber tube which expands against a hole through which the fluid must pass. By this means the uniformity of flow is not affected by the swinging, stretching, or other manipulation of the conducting tube.

The asepsis of the injection procedure is maintained by a completely closed system, entirely independent from the pumping mechanism. This closed system

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is sterilizable in the autoclave, since it consists of a single tube from beginning to end without connections of any kind.

The recipient is relieved of the duty of keeping his arm in one position by a combination of three features. The first has already been mentioned: liquid will be discharged uniformly in spite of all manipulations of the conducting tube. The second is that the tube is so firmly anchored to the arm by a substantial arm plate (Fig. 3) that it cannot be dislodged. The third is that the

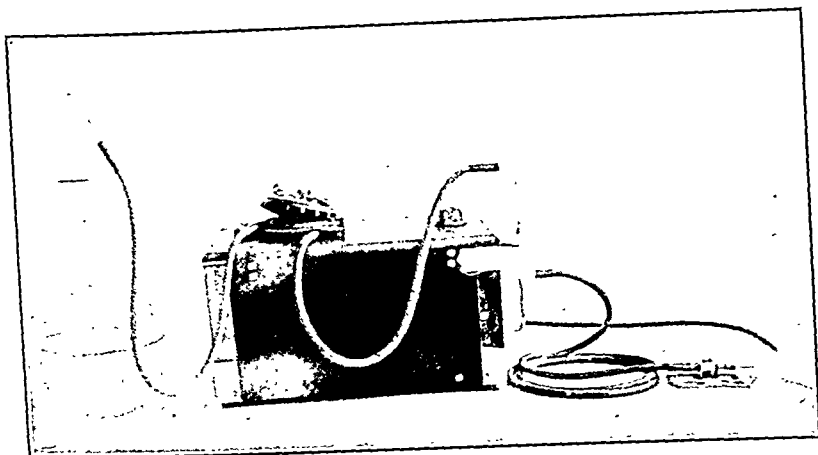


Fig. 1.—Photograph of the complete apparatus.

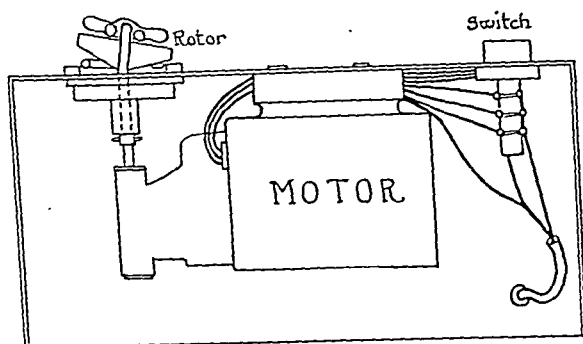


Fig. 2.—Sketch of the mechanism.

liquid is injected into the vein through a fine rubber capillary tube which is so flexible that no movements of the arm can affect its position within the vein. Not only may the recipient move his arm as he pleases, but also is only little disturbed by the capillary tube in his vein.

When the injection is prolonged and the exchange of supply flasks frequent, a connection such as that shown in Fig. 1 is useful. It consists of a glass tube to withdraw the liquid and a sealed-on glass skirt to enclose the neck of the flask. This connection insures sterility, facilitates refilling, and permits the flasks to stand upright.

If the operator wishes that a signal be given when the supply flask has been emptied to a certain level, he may set the flask upon one pan of a balance which

has a counterpoise on the other pan representing the weight of the flask when it has been emptied to this level. Further withdrawal of liquid will then cause the balance to swing and close an electric circuit containing a suitable signal. He may also interpose a trap in the conducting tube to catch air and sediment and to indicate the rate of flow.

The technic used to operate this apparatus is as follows: After sterilization, the conducting tube is attached to the machine and the machine set in motion. When all the air has been driven out of the tube and liquid has begun to flow regularly from the release valve, an intravenous needle (Size 15) is placed in

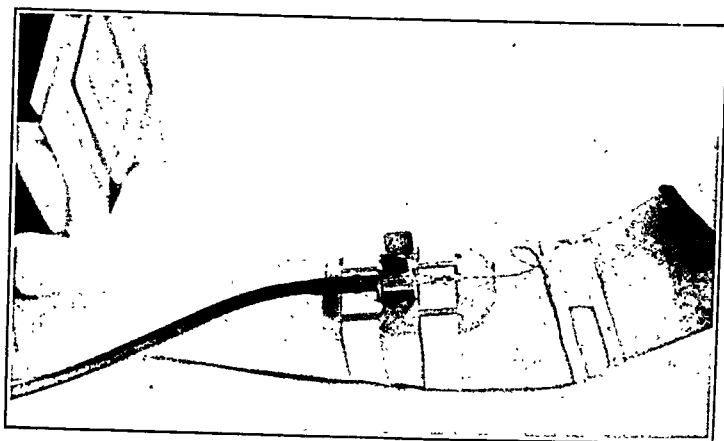


Fig. 3.—Photograph of arm of recipient with apparatus attached.

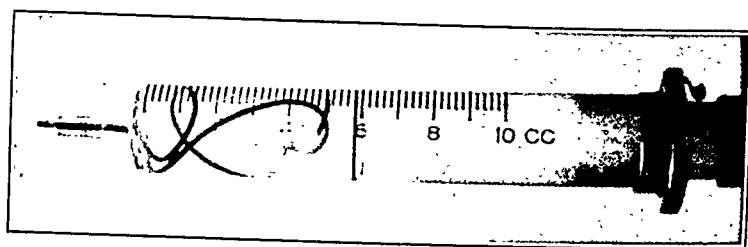


Fig. 4.—Photograph of a "threaded" syringe.

the vein. The capillary rubber tube is injected through the needle from a syringe (Fig. 3). The knot in the proximal end of the capillary tube, which was placed there to catch at the shoulder of the syringe, is cut off after the syringe has been detached, and then the needle is withdrawn. The capillary tube is attached to the release valve through a fine Luer needle. The release valve is attached to the arm plate after the latter has been taped to the arm. A small dressing is placed over the puncture wound through which the tube passes. The injection may then be continued indefinitely, the recipient meanwhile having the liberty to move his arm as he pleases. The rate of injection may be changed by the ratios of 1:2:4 by turning a switch. The present machine delivers liquid at the rates of 75, 150, and 300 c.c. an hour. Rates outside these limits require the use of tubes of suitable size.

Because the discharge into the vein is slow and constant the liquid need not be warmed. For the same reason hypertonic solutions of glucose (in normal salt solution) may be injected safely.

The several mechanical features in this apparatus which contribute to its successful performance are the following: The driving mechanism is completely enclosed and will run indefinitely without attention. The pump will develop enough pressure to burst the tube, yet the wear on the tube by the rotor is negligible. The release valve is made of stainless steel and furnishes, through connection to the arm plate, absolute anchorage. All parts susceptible to deterioration, namely, the conducting tube, the rubber capillary tube, and the tube in the release valve, are readily and cheaply replaceable. Clotting within the capillary tube is impossible, because blood has no opportunity to enter it, since liquid is continuously flowing out. A smooth and uniform discharge from the capillary tube is guaranteed by a balance of two forces, the cushioned liquid pressure within the conducting tube and the resistance of the release valve. The rubber capillary tube, which was developed especially for this work, has an outside diameter of 0.04 inch. It is made of high grade anode rubber, and it will retain its elasticity and tensile strength through many days of contact with blood.

The Oak Manufacturing Company, Chicago, Illinois, kindly undertook to make this machine.

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## THE STABILITY OF DIOTHANE SOLUTIONS\*

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ATTENTION has already<sup>1</sup> been called to the unusual behavior of some phenyl urethane local anesthetics when their solutions are boiled. The monophenyl urethanes of di-n-butyl- and di-iso-butyl-amino propanediols were found to be altered by boiling their solutions in such a way that their activity as surface anesthetics materially increased while the anesthesia produced by their intradermal injection was shortened. This was interpreted as being due to some change of an unknown nature which resulted in the creation of a more rapidly diffusible form of anesthetic.

When diothane (piperidinopropanediol di-phenyl urethane hydrochloride) was selected as a new and valuable anesthetic for clinical trial, the behavior of its solutions on heating was checked, and it was found and reported that sterilization over prolonged periods did not alter the solution according to physiologic tests.<sup>2</sup>

A further detailed chemical study of diothane and its solutions has revealed the fact that the anesthetic free base is relatively unstable to heat. Heat-

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\*From the Research Laboratories of The Wm. S. Merrell Company.  
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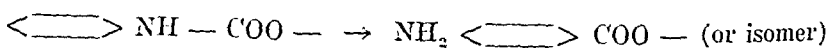
ing this free base to its decomposition point in a vacuum has yielded traces of aniline together with other unknown decomposition products. The crystalline hydrochloride is quite stable to moderate heat (100° C.) when the crystals are dry, but somewhat unstable to heat when they are wet with water or other solvent capable of partially dissolving them. The amount of change is too slight to allow the isolation of any decomposition products.

Since the wet crystals proved to be somewhat unstable it was deemed advisable to restudy the stability of diothane solutions.

The treatment of local anesthetic solutions with dilute nitrous acid followed by the addition of an alkaline solution of beta-naphthol is a recognized test for distinguishing those products containing primary amino groups. Thus procaine gives a dark brick-red precipitate (positive) while cocaine gives a yellow-white precipitate (negative). Freshly prepared solutions of pure diothane crystals give a typical negative reaction. However, when these solutions are heated for prolonged periods (> one hr. at 98° C.) or aged for many months, diazotization and coupling with beta-naphthol gives a light orange precipitate.

This change as attested by the delicate chemical reaction is accompanied by no demonstrable change in anesthetic activity or toxicity of the solution, indicating that the extent of any change is extremely slight.

Theoretical consideration of possible changes which would result in the creation of diazotizable amino groups suggested only two reactions. The first of these was a possible rearrangement as follows:



Since this rearrangement would account for the formation of a colored dye in the test reaction, an attempt was made to determine the amount of change which would account for the degree of color actually developed. In order to check this point to some extent diothane solutions were made up containing various quantities of procaine, and these solutions were tested together with the oldest diothane solution available (two years). The colors developed by the solution containing procaine were quite different in shade, but the intensity of color developed by the old diothane solution was similar to that of a fresh diothane solution containing procaine 1:90,000. Similar tests were carried out by adding traces of the di-p-amino benzoate homologues of diothane, and since these products contain two primary amino groups per molecule even smaller proportions were required to give colors of the same intensity.

In this connection it is of interest that the di-p-amino benzoate of piperidinopropanediol<sup>3</sup> has almost no activity as a mucous membrane anesthetic. It is obvious, therefore, that if this product were found in diothane solutions in any quantity, the change would be detectable by physiologic tests.

Remembering that thermal decomposition of diothane free base had yielded traces of aniline, diothane solutions with added quantities of aniline hydrochloride were also tested in comparison with the oldest diothane solution. Again the shade of color developed in the test was somewhat different, but an even closer comparison of intensities was possible. In this case, the intensity de-



veloped with the old diothane solution was most closely matched by that with a fresh diothane solution to which aniline had been added in a concentration of 1:200,000.

In both cases the color developed by the diothane solution, to which the primary amino compounds had been added, was concentrated on the otherwise yellow-white precipitate, making the very slight color formation quite obvious. In neither case was there similarity enough in the shade of color to indicate that either an amino benzoate or aniline had actually been formed. These experiments indicate, however, that an extremely small proportion of the diothane had been altered.

While in the above discussion the two-year-old diothane solution was specifically mentioned, the same alteration of the solution may be rapidly achieved by heating the solution for several hours on a steam-bath. The addition of a slight excess of acid to the solution markedly delays such changes as take place, and no significant change occurs during the first hour of heating, so that solutions may be adequately sterilized without alteration.

Attempts to correlate the changes in diothane solutions with the increased surface activity of the di-n-butyl- and di-iso-butyl-amino propanediol mono-phenyl urethane solutions have not been productive, since these boiled solutions do not yield any colored product on attempted diazotization and coupling with beta naphthol.

#### SUMMARY

Prolonged heating or aging of diothane solutions produces a very slight degree of alteration detectable by delicate colorimetric testing, but not detectable by pharmacologic methods. Theoretical considerations of possible changes have been discussed, and it has been inferentially shown that the concentration of any alteration product in diothane solutions is probably not greater than 1:100,000.

The writer wishes to acknowledge the assistance of Dr. E. W. Scott and Messrs. A. R. Lux and Karl Bambach.

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## THE PRESERVATION OF BIOLOGIC SPECIMENS\*

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**T**ISSUES which are to be preserved for study or exhibit are often fixed by means of a formalin solution, and then kept immersed in an aqueous solution of sodium acetate containing glycerin and thymol. It is a common observation that the preserving media become clouded after a few weeks or months and must be replaced. To reduce the necessity for frequent replacement, we have undertaken a study of the cause of clouding, hoping to be able to control it by suitable means.

Our investigations have shown the turbidity, which seems not to be peculiar to any special kind of tissue although some specimens induce more rapid clouding than others, is due to oxidation of the thymol in the preserving fluid. While thymol is an inhibitor of bacterial growth, its solubility in water is low, and there seemed to be a possibility of bacterial changes causing precipitation in our solutions. This possibility has been definitely disproved as will be shown later.

### METHODS OF STUDY

In these studies, we have used the solutions already referred to. Tissues known by experience to have a tendency to cause clouding were fixed by soaking in a solution of the following composition: Chloral hydrate 500 gm., sodium chloride 180 gm., sodium bicarbonate 100 gm., sodium sulphate 220 gm., 40 per cent formalin solution 500 c.c., water to make 10,000 c.c. After the tissues had been hardened in this solution for several days, they were washed in running water for twenty-four hours, and then, after suitable mounting, kept immersed in a solution consisting of glycerine 2,000 c.c., sodium acetate 800 gm., thymol 100 gm., and water to make 10,000 c.c. The thymol was dissolved in boiling water, added to the main bulk of solution, and the whole cooled and filtered before using. Considerable excess thymol was removed in this step. Where special solutions were needed, they were made up in small amounts, and their use is reported below.

The tissues used for our controlled experiments were from a normal brain, and showed a marked tendency to induce clouding.

Since clouding of our media did not set in normally until six weeks or more after they had been prepared, we heated some of our specimens to a temperature of about 50° C., so that precipitation would be observed in a matter of days rather than weeks. The conclusions to be drawn from the experiments at the higher temperatures can with due allowance be applied to conditions at room temperature.

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## RESULTS AND INTERPRETATION

The first clue to the cause of clouding was found in the observation that all specimens led to turbidity, and that where it occurred the turbidity began after several weeks' standing and then increased slowly to a maximum. The white cloudiness seemed to be of the same character in all cases and resembled that noted when saturated thymol solutions are cooled.

It was thought that some obscure bacterial infection was the cause of the difficulty. Were there bacterial action operating, however, the clouding would probably develop quickly and continuously. In an endeavor to observe the actual organisms present, clouded fluid was examined under a microscope. It appeared perfectly clear. This indicates that the precipitate is very finely divided, but does not exclude the possibility of very small microorganisms being present. All efforts to culture the clouded media failed. Inoculation of a new sample of the preserving fluid with clouded material failed to accelerate the development of new cloudiness. These observations indicated the absence of bacterial factors in this phenomenon.

It became evident to us that some slow chemical change in the medium itself, perhaps influenced by material extracted from the biologic specimens, is responsible. Two simple observations convinced us that this is the case, and that the source of the clouding is the thymol in the preserving medium. First, a badly clouded solution in which a panophthalmia exhibit had been kept was clarified by repeated boiling with paper fibers, and filtration. It was returned to the original jar containing the specimen and showed no further precipitation over a period of four months. On adding thymol to this solution, slight clouding was noted after four weeks. Second, a sample of the usual medium alone showed a turbidity after standing in the laboratory for from six to eight weeks.

The precipitate, we noted, is insoluble in acid and alkali, but is quickly dissolved by ether. It is not coagulated by strong calcium chloride solution. This behavior conforms to the expected action of thymol or a thymol derivative. The precipitate, we noted further, always begins at the surface of the preserving solution, indicating oxidation as a possible factor. This was confirmed in the following way. A sample of the original medium was slightly diluted with water and kept at 50° C. A second sample was simultaneously diluted with hydrogen peroxide solution, and kept at the same temperature. The latter sample clouded in a few hours, the former did not. It appeared, therefore, that oxidation has some part in the phenomenon. The experiment was repeated at room temperature. The medium with peroxide clouded in four days, the control medium has remained clear for as many weeks.

To determine whether the biologic samples themselves contribute to this oxidation, we kept a sample of tissue under water at 50° C., for several days. Naturally there was considerable extraction of soluble material in this period. The water extract was filtered and added to some preserving medium. A precipitate developed in a matter of a few days, where otherwise, it would not. Some water soluble material from the tissues, therefore, must catalyze the formation of the observed precipitate. Whether there may be other reactions than oxidation of the thymol which contribute to the clouding, our experiments do not show. We believe, however, that this is likely.

## AN IMPROVED PRESERVING FLUID

While these observations have identified thymol as the cause of precipitation in our fluids, there remained the problem of finding an adequate substitute. Certainly replacement of the thymol seems called for. A tissue placed in preserving medium without thymol has remained indefinitely clear, but there is some danger of mould growth in an acetate solution not protected by a fungicide. We have found that a small amount of phenol or toluol added to the solution gives the desired protection. Such solutions have remained clear for a period of nearly six months.

We have tried other methods of preservation, most of which have proved unsatisfactory. A specimen kept under pure glycerin became rather badly discolored after a short time. A thin coating of collodion did not prevent clouding of one specimen, but a coating of varnish did. Had the collodion film been thick and air-dried more thoroughly, it would undoubtedly have further slowed up the clouding, but would not eventually preclude its appearance. The coating of varnish, likewise, does not offer permanent protection against clouding.

A layer of paraffin oil over the acetate preserving solution, by slowing up the admission of oxygen, inhibited but has not prevented clouding of the medium. When immersed in pure paraffin oil and maintained at 50° C. for several weeks, one specimen caused no clouding, but on cooling there was developed a heavy turbidity, which disappears on reheating. This probably is due to water which was distilled into the paraffin at the higher temperature, and which was thrown down by cooling. At constant temperature, however, there is no precipitate and if kept at room temperature, paraffin oil should be a satisfactory preserving medium. By actual test, we have found the oil with tissue immersed in it to remain clear for over three months. Substitution of magnesium sulphate in equivalent concentration for the sodium acetate does not greatly retard the formation of cloudiness; it had been thought that the higher ionic charge would decrease the formation of the colloidal suspension.

## SUMMARY AND CONCLUSIONS

1. An investigation of the media in which biologic specimens are preserved shows that the frequently observed clouding is due to a chemical oxidation and precipitation of thymol in the preserving fluid, catalyzed by materials extracted from the tissues.

2. An improved fluid in which phenol or toluol is substituted for thymol has proved satisfactory in practice.

3. Paraffin oil may be used as a preserving fluid, provided the temperature does not vary over too wide a range.

The assistance of Dr. M. J. Fein, pathologist of the Cumberland Hospital, in carrying out this work is acknowledged.

# CHANGES IN CELL VOLUME PRODUCED BY VARYING CONCENTRATIONS OF DIFFERENT ANTICOAGULANTS\*

V. G. HELLER, PH.D., AND HENRY PAUL, B.S., STILLWATER, OKLA.

A STUDY of the distribution of certain inorganic elements in cells and plasma reveals that the cell-plasma ratio varies not only with the nature of the anticoagulant but also with its concentration. Since the accuracy of such an investigation is dependent upon a definite cell volume as a basis for each calculation, the literature was investigated to ascertain the preferable method of procedure. Many substances are recommended as anticoagulants, the kinds, concentrations, and manner of use being almost as variable as the number of investigators. Definite lengths of time and speed of centrifuging, as a rule, are not stated. Guillaumin,<sup>1</sup> Eisenman,<sup>2</sup> and Hirota<sup>3</sup> all give some data pertaining to the subject in general but fail to furnish information applicable to this problem. In many cases, apparently, the manner of determining the cell volume was largely a matter of choice, so far as any definite method of procedure was concerned. For these reasons, it was deemed advisable to investigate the effects of the commonly recommended anticoagulants, and the conditions most suitable for determining the true cell volume.

## EXPERIMENTAL

Of the anticoagulants commonly recommended, the following have been employed in this investigation; sodium, potassium, and ammonium oxalates of concentrations 0.1 to 0.4 per cent by weight, the corresponding citrate salts of concentrations 0.2 to 0.8 per cent by weight, and heparin of concentrations 0.1 to 1 mg. per ml. of blood.

The cell volumes were determined using 4 mm. bore hematocrit tubes 15 cm. in length. The cell and serum heights in the tubes were measured with vernier calipers, thus eliminating the use of graduated tubes. The tubes were filled by means of capillary tubing; after which they were sealed and suspended on a rubber cushion in a position which would bring their axes perpendicular to the axis of rotation while centrifuging. A large size standard International Centrifuge was used, the speed being checked by use of a tachometer.

The time required at various speeds for the cell volume to become constant was ascertained. Determinations were made at two speeds—1800 and 2200 R.P.M. At lower speeds the time required was excessive, and it was difficult to prevent hemolysis; at higher speeds the tubes were frequently broken. The apparent cell volume was read at intervals during the centrifuging, and the data so obtained are presented in the accompanying chart. An examination of these curves reveals that in the case of cow's blood the cell volume becomes constant in 65 minutes at 1800 R.P.M. and in 45 minutes at

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2200 R.P.M. while for chicken blood, which contains larger cells, only 40 minutes at 1800 R.P.M. were required.

Using these standard conditions the cell volumes were determined with the various anticoagulants previously mentioned. Blood of cattle, chickens, and rats was drawn with an hypodermic needle, either from the jugular vein or by heart puncture, and delivered into flasks coated with the designated quantity of anticoagulant. After thorough mixing and chilling, the blood was delivered to the hematocrit tubes as described. The concentrations mentioned earlier in this article represent the extremes used by most investigators. These tests have been frequently repeated for various types of blood, and typical results are shown in Table I.

It will be observed from an inspection of the table that the ammonium salts produce an increase in cell volume with an increase in the percentage of

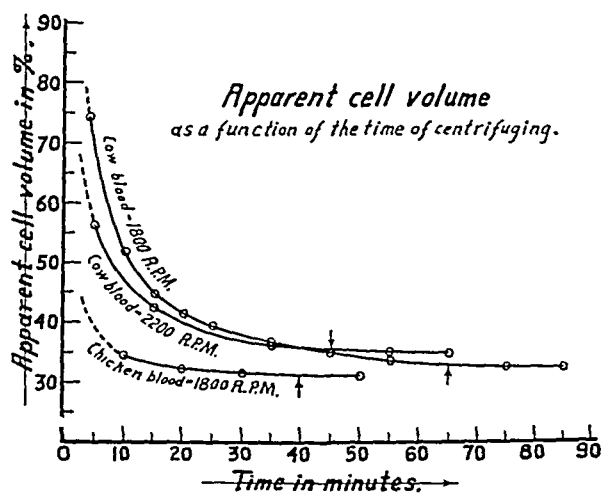


Chart 1

anticoagulant used, while sodium, potassium, and lithium salts produce a cell-volume decrease with percentage increase of salts used. Various determinations indicate that about 0.2 per cent of the oxalates and 0.4 per cent of the citrates produce the most favorable results from the standpoints of anticoagulation and hemolysis. Since ammonium salts produce an increase in cell volume and the other salts have an opposite effect, various mixtures have been tested, and it has been found that with the use of an 0.2 per cent concentration of a mixture of 40 per cent potassium oxalate and 60 per cent ammonium oxalate there is a minimum change in cell volume. These data are presented in Table II.

In the analysis for inorganic constituents, any inorganic anticoagulant interferes. Heparin was tried for this reason although it carries a calcium contamination. Its use is more difficult than that of the inorganic compounds as it does not dissolve as readily due to its dryness, and there is, consequently, a tendency to form small clots about the particles of heparin. It cannot be dissolved and evaporated over the surface of the container as it is an organic

TABLE I

CHANGES IN CELL VOLUME WITH VARYING CONCENTRATIONS OF ANTICOAGULANTS

| OXALATES          |                 |        |                 | CITRATES          |                 |        |                 |
|-------------------|-----------------|--------|-----------------|-------------------|-----------------|--------|-----------------|
| ANTICOAGULANT     | CELL VOLUME     |        | SOURCE OF BLOOD | ANTICOAGULANT     | CELL VOLUME     |        | SOURCE OF BLOOD |
|                   | AMOUNT PER CENT | CHANGE |                 |                   | AMOUNT PER CENT | CHANGE |                 |
| Potassium oxalate |                 |        | Cow             | Potassium citrate |                 |        | Rat             |
| 0.1%              | 33.33           |        |                 | 0.2%              | 34.20           |        |                 |
| 0.2%              | 32.20           | -1.13  |                 | 0.4%              | 32.60           | -1.60  |                 |
| 0.3%              | 31.02           | -1.18  |                 | 0.6%              | 31.20           | -1.40  |                 |
| 0.4%              | 30.22           | -0.80  |                 | 0.8%              | 30.10           | -1.10  |                 |
| Sodium oxalate    |                 |        | Cow             | Lithium citrate   |                 |        | Cow             |
| 0.1%              |                 |        |                 | 0.2%              | 32.33           |        |                 |
| 0.2%              | 32.42           |        |                 | 0.4%              | 31.20           | -1.33  |                 |
| 0.3%              | 31.22           | -1.20  |                 | 0.6%              | 29.85           | -1.35  |                 |
| 0.4%              | 29.70           | -1.52  |                 | 0.8%              | 29.10           | -0.75  |                 |
| Ammonium oxalate  |                 |        | Chicken         | Ammonium citrate  |                 |        | Chicken         |
| 0.1%              | 31.34           |        |                 | 0.2%              | 28.81           |        |                 |
| 0.2%              | 32.65           | +1.31  |                 | 0.4%              | 29.85           | +1.04  |                 |
| 0.3%              | 33.30           | +0.75  |                 | 0.6%              | 31.98           | +2.13  |                 |
| 0.4%              | 33.80           | +0.50  |                 | 0.8%              | 33.97           | +1.99  |                 |
| Ammonium oxalate  |                 |        | Cow             |                   |                 |        |                 |
| 0.1%              | 39.66           |        |                 |                   |                 |        |                 |
| 0.2%              | 40.82           | +1.16  |                 |                   |                 |        |                 |
| 0.3%              | 41.54           | +0.72  |                 |                   |                 |        |                 |
| 0.4%              | 41.80           | +0.26  |                 |                   |                 |        |                 |

TABLE II

THE EFFECT OF A MIXTURE OF POTASSIUM OXALATE AND AMMONIUM OXALATE ON CELL VOLUME

| ANTICOAGULANT                                     | CELL VOLUME     |        | SOURCE OF BLOOD |
|---------------------------------------------------|-----------------|--------|-----------------|
|                                                   | AMOUNT PER CENT | CHANGE |                 |
| 40% Potassium oxalate and<br>60% Ammonium oxalate |                 |        | Rat             |
| 0.1%                                              | 41.2            |        |                 |
| 0.2%                                              | 41.2            | 0      |                 |
| 0.3%                                              | 41.1            | -0.1   |                 |
| 0.4%                                              | 41.0            | -0.1   |                 |

compound, which might decompose on heating. The most satisfactory procedure consisted in making a water suspension, which caused the heparin to dissolve rapidly when added to the blood. Although the use of this compound as an anticoagulant has been repeatedly tried, it is impossible to secure results that check as favorably as those for the inorganic compounds. Table III presents the averages of two series of results from the experimental use of heparin to coagulate the blood of rats and of chickens. These data indicate

TABLE III

CELL VOLUME PRODUCED BY VARYING CONCENTRATIONS OF HEPARIN  
AS COMPARED TO POTASSIUM OXALATE

(MG. PER ML.)

| SOURCE OF BLOOD | K <sub>2</sub> C <sub>2</sub> O <sub>4</sub> | HEPARIN |       |       |       |
|-----------------|----------------------------------------------|---------|-------|-------|-------|
|                 | 2                                            | 0.1     | 0.2   | 0.4   | 1.0   |
| Rat             | 45.22                                        | 44.23   | 43.70 | 42.00 |       |
| Chicken         | 27.00                                        | 26.19   | 26.19 | 26.16 | 26.37 |

that the concentration does not affect the ratio noticeably, but there is some cell packing with greater concentration. The cell volume is always slightly less than when inorganic anticoagulants are used.

#### CONCLUSIONS

1. There is a need for a specified concentration of anticoagulant as well as speed and time of centrifuging if cell-serum ratios are to be used in calculations.
2. Increasing concentrations of sodium, potassium, or lithium salts cause a decrease in cell volume.
3. Ammonium salts cause an increase in cell volume with increased concentration.
4. Within normal limits, a mixture of 40 per cent potassium oxalate and 60 per cent ammonium oxalate gives a minimum cell volume variation.
5. A speed of 2200 revolutions per minute was found most satisfactory, all factors being considered.

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### THE KOLMER, KAHN, AND MEINICKE REACTIONS IN THE TROPICS\*

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THE object of this study was to adopt for our laboratories a test which can be rapidly performed, is of highest sensitivity, is of greatest technical accuracy, and which is specific in its reaction, uniform in results, and also simple and economical.

#### MATERIAL

1. Serums from 700 syphilitic cases included primary, secondary, and tertiary congenital and latent syphilis. One-third of the cases presented secondary syphilis. Most of the patients were untreated.

2. Serums of 300 nonsyphilitic controls were obtained from 100 patients with severe secondary anemia due to intestinal parasites; from fifty cases of acute malarial fever; from fifty cases of chronic malaria; from twenty-five cases of mycotic disease of the skin; from 10 lepra cases; fifteen frambesias and fifty postoperative cases. None of these patients presented any symptoms or gave any history of syphilis. Careful observations were made on all cases; complete physical examinations and histories were taken in all.

\*From the Laboratories of the Santa Clara Hospital and Departments of Pathology and Parasitology of the University of Cartagena.

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TABLE I  
RESULTS OBTAINED AND METHODS USED IN 700 CASES OF SYPHILIS

| METHODS                     | NUMBER OF POSITIVE REACTIONS | NUMBER OF DOUBTFUL REACTIONS | NUMBER OF NEGATIVE REACTIONS |
|-----------------------------|------------------------------|------------------------------|------------------------------|
| Kolmer Complement Fixation  | 515                          | 5                            | 180                          |
| Kahn Standard Precipitation | 525                          | 15                           | 160                          |
| Meinicke Clarification      | 520                          | 12                           | 168                          |

TABLE II  
RESULTS OBTAINED IN 300 NONSYPHILITIC CONTROLS

| NUMBER OF CASES                                      | NAME OF DISEASE                      | KAHN     |          | KOLMER   |          | MEINICKE |          |
|------------------------------------------------------|--------------------------------------|----------|----------|----------|----------|----------|----------|
|                                                      |                                      | POSITIVE | DOUBTFUL | POSITIVE | DOUBTFUL | POSITIVE | DOUBTFUL |
|                                                      |                                      | +        | +        | +        | +        | +        | +        |
| 100                                                  | Secondary Anemia of Parasitic Origin | 2        | 4        | 0        | 0        | 2        | 3        |
| 50                                                   | Acute Malaria                        | 1        | 2        | 0        | 1        | 1        | 4        |
| 50                                                   | Chronic Malaria                      | 1        | 2        | 0        | 0        | 1        | 2        |
| 25                                                   | Mycosis of Skin                      | 0        | 2        | 0        | 0        | 1        | 3        |
| 10                                                   | Leprosy                              | 1        | 1        | 0        | 1        | 1        | 2        |
| 50                                                   | Postoperative Cases                  | 1        | 2        | 0        | 0        | 4        | 4        |
| 15                                                   | Frambesia                            | 15       | 0        | 15       | 0        | 15       | 0        |
| Total                                                |                                      | 6        | 13       | 0        | 2        | 10       | 20       |
| All methods gave 100 per cent positives in frambesia |                                      |          |          |          |          |          |          |

## COMMENT

The Kolmer complement fixation test is harder to perform and less economical than the precipitation tests of Kahn and Meinicke, but the results obtained are of greater uniformity and give the lowest percentage of non-specific doubtful reactions. In our group of negative serums, we had not one nonspecific positive reaction with the Kolmer test. Even though it is a more complicated test to learn and harder for the technician to perform, yet once mastered, the test is much more easily interpreted than the precipitation tests. It gives clear negatives in every type of the tropical diseases in which we have used it, when there was no manifestation of syphilis involved. In the repeated tests of the same serums the Kolmer test gave the same reactions.

The Kahn test, though of greatest sensitivity, is less specific in reaction than the complement fixation test. However, the simplicity of the test and the rapidity with which it can be done recommend it for daily routine in the laboratories of tropical countries. The Kahn test is as hard to interpret as it is easy to perform. It requires skill and good eyesight to avoid false reactions. Doubtful reactions should always be repeated in conjunction with the complement fixation test. This test is of great value in emergencies as an immediate aid in the diagnosis.

The Meinicke test is less sensitive than the Kahn and gives the greatest percentage of nonspecific positive reactions in our group. It is very easy to perform and the interpretation, when delayed for several hours, is much easier

than in the Kahn test. When read immediately, the same difficulties are met. Our technicians prefer the Meinicke test to other precipitation tests because of the easier interpretation.

In all laboratories the precipitation tests should be always performed in conjunction with the complement fixation test. In the tropics, a precipitation test is of great value because of the rapidity with which it can be performed. The climate tends to decompose the blood very rapidly, and often it is necessary to perform the test as soon as specimens reach the laboratory. All doubtful and suspected positive reactions are always repeated in conjunction with the complement fixation tests. For specificity, we depend on the Kolmer test.

#### CONCLUSIONS

1. One thousand serums have been studied by the Kolmer, Kahn and Meinicke reactions; 700 serums were from known syphilitic cases, and 300 were negative controls.

2. The precipitation tests were found most sensitive, but less specific than the complement fixation test.

3. The precipitation tests are less complicated to perform and more economical, but they are harder to interpret than the complement fixation test.

4. The precipitation tests are readily adaptable in routine laboratory procedure; because of the rapidity with which the test may be performed, it is of value in emergencies.

5. The Kolmer complement fixation test is more complicated to perform, but easiest to interpret. It is most specific in reaction.

6. The precipitation tests should always be used in conjunction with the complement fixation test, except in emergency cases.

I am grateful to Dr. Kolmer and Dr. Kahn for supplying me with antigens for this study.

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# NOTE ON THE DETERMINATION OF BLOOD UREA BY DIRECT NESSLERIZATION OF A SODIUM TUNGSTATE—SULPHO- SALICYLIC ACID FILTRATE\*

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THERE are several methods of determining urea by direct nesslerization of a protein-free or nearly protein-free blood filtrate. Karr,<sup>2</sup> Roe and Irish,<sup>5</sup> Myers,<sup>4</sup> and Looney<sup>3</sup> all have described methods based on the nesslerization of a Folin and Wu<sup>1</sup> tungstic acid filtrate of blood. None of these methods has been entirely satisfactory for routine procedures. The aeration method of Myers<sup>4</sup> is simple and, if relatively large numbers of determinations are being run simultaneously, is very satisfactory. If only an occasional urea determination is made, or if speed is of importance, either the aeration method or the distillation method requires too much time.

Woodward and Fry<sup>6</sup> have recently used sulphosalicylic acid for the preparation of an acid, protein-free blood filtrate for the determination of blood glutathione. This reagent seemed to offer possibilities as a protein precipitant for the direct determination of urea in blood.

## EXPERIMENTAL

Woodward and Fry used one volume of an approximately molar solution of sulphosalicylic acid for the precipitation of the proteins of one volume of blood after dilution with eight volumes of water. They found that clear protein-free solutions could be obtained with human blood. In the experiments reported here, it has been found that some bloods, particularly swine blood, gave a brownish colored filtrate when sulphosalicylic acid alone was used. Other bloods usually gave clear, colorless filtrates. By including sodium tungstate in the precipitating mixture, the yield of filtrate was smaller but, with all bloods examined, a colorless filtrate was obtained. These filtrates were found to develop a brownish color when made alkaline to litmus. The further addition of alkali resulted in a perfectly clear, colorless solution. The amount of alkali in 2 ml. of a dilute solution of Bock-Benedict Nessler's reagent was found to discharge completely the brown color of 5 ml. of neutralized blood filtrate. Nesslerized solutions, with or without ammonia present, would remain clear for several hours.

### *Procedure for the Determination of Urea in Blood.—*

#### Solutions:

1 M sulphosalicylic acid; 25 gm. of sulphosalicylic acid in 100 ml. of solution (Woodward and Fry<sup>6</sup>).

10 per cent sodium tungstate solution (Folin and Wu<sup>1</sup>).

\*From the Department of Veterinary Research, Iowa State College.  
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Urease solution of Folin and Wu mixed just before using with the activating phosphate solution in a ratio of 9:1.

Bock-Benedict Nessler's solution (Myers').

Nitrogen standard. 0.0472 gm.  $(\text{NH}_4)_2\text{SO}_4$  per liter, 5 ml. 0.5 mg.

Sodium hydroxide approximately  $2/3$  N.

Technic:

Two milliliters of oxalated blood (2-3 mg. of potassium oxalate per milliliter of blood) and 1 ml. of fresh urease solution are pipetted into a large test tube or a small Erlenmeyer flask, the contents mixed, and the container stoppered and placed in a water-bath at 50 degrees C. for fifteen minutes. To the tube is added 13 ml. of distilled water, 2 ml. of sodium tungstate solution and with constant agitation, 2 ml. of the sulphosalicylic acid solution. The material is then filtered through a fairly retentive filter paper.

Five milliliters of filtrate is pipetted into a 25-ml. graduate cylinder. If a high urea content is anticipated, 5 ml. or more of water may be added; otherwise 1 ml. of  $2/3$  N sodium hydroxide is pipetted into the solution followed immediately by 2 ml. of dilute Nessler's reagent. The solution may be diluted further to approach the color intensity of 0.5 mg. of ammonia nitrogen in the standard.

The calculation then is:

$$\frac{S}{R} \times 0.5 \times \frac{D}{100} \times \frac{100}{0.5}$$

TABLE I  
RECOVERY OF ADDED UREA

| SAMPLE      | MG. UREA N ADDED | MG. UREA N FOUND | PERCENTAGE RECOVERY |
|-------------|------------------|------------------|---------------------|
| 2 ml. blood | 0.000            | 0.107            | ----                |
| 2 ml. blood | 0.194            | 0.300            | 99.4                |
| 2 ml. blood | 0.388            | 0.500            | 101.3               |

TABLE II

COMPARISON OF THE SSA DIRECT AND AERATION METHODS OF DETERMINING UREA NITROGEN

| SAMPLE  | SPECIES | MG. PERCENTAGE N |      | PERCENTAGE DIFFERENCE |
|---------|---------|------------------|------|-----------------------|
|         |         | AERATION         | SSA  |                       |
| 1       | Swine   | 12.2             | 11.8 | -3.3                  |
| 2       | Swine   | 15.0             | 15.0 | 0.0                   |
| 3       | Swine   | 16.3             | 15.7 | -3.6                  |
| 4       | Swine   | 11.6             | 12.5 | 7.8                   |
| 5       | Swine   | 11.9             | 12.3 | 3.3                   |
| 6       | Swine   | 10.6             | 10.8 | 1.8                   |
| 7       | Ox      | 10.4             | 10.0 | -3.8                  |
| 8       | Ox      | 13.0             | 13.5 | 3.9                   |
| 9       | Ox      | 12.0             | 12.0 | 0.0                   |
| 10      | Horse   | 8.5              | 8.6  | 1.2                   |
| 11      | Horse   | 9.0              | 9.1  | 1.1                   |
| 12      | Horse   | 7.4              | 7.5  | 1.4                   |
| 13      | Sheep   | 17.3             | 16.7 | -3.4                  |
| 14      | Dog     | 10.7             | 11.3 | 5.6                   |
| 15      | Dog     | 9.5              | 9.0  | -5.2                  |
| 16      | Human   | 13.5             | 13.0 | -3.7                  |
| Average |         | 11.8             | 11.8 | 0.0                   |

*Effect of the Sodium Salt of Sulphosalicylic Acid on Nesslerized Solution.*—Two nitrogen standards containing 1 mg. N were prepared; to one was added a neutralized solution of 5 ml. of M sulphosalicylic acid. After nesslerization and dilution to 100 ml., the two solutions gave identical intensity of color in the colorimeter.

*Recovery of Added Urea.*—Aliquot portions of a blood sample were measured into tubes. Water was added to the control, and urea solution to the other two tubes in order to keep all volumes equal. The results are shown in Table I.

Blood urea nitrogen of various species of animals was determined by the direct method as described, and simultaneous determinations were made by the aeration method. These samples were obtained from different sources, and nothing is known regarding the health of the animals. The comparative data are shown in Table II.

#### DISCUSSION

In the precipitation of the proteins of blood and urease, it has been found necessary to use both sodium tungstate and sulphosalicylic acid in order to get a colorless filtrate satisfactory for nesslerization. The filtrate obtained by using sulphosalicylic acid only was sometimes very brown, having almost the color of a nesslerized solution. Although this color was discharged by making the solution decidedly alkaline, the use of the sulphosalicylic acid and sodium tungstate combination was found preferable.

The alkalinity of the final solution is very important. Too high a concentration of alkali results in a turbid solution. It would be satisfactory to add the sodium hydroxide to the Nessler solution provided that sufficient amounts of acid were also added to the standard. Satisfactory results have been obtained by adding the sodium hydroxide after the Nessler's solution, instead of before as previously stated.

Fowl blood does not lend itself to the determination of urea by this method. The blood is low in urea, and it is very difficult to obtain samples free of clots. Cloudy solutions, which may be due, at least in part, to the high concentration of oxalate necessary to prevent coagulation, have frequently resulted.

Woodward and Fry<sup>6</sup> state that too much oxalate prevents complete precipitation of proteins with sulphosalicylic acid. The same has been found for citrates and fluorides; however, the amounts generally used as anticoagulants cause no difficulties.

#### SUMMARY

A simple method for the direct determination of urea in blood has been described.

#### ADDENDUM

Because of unavoidable circumstances the paper of Taylor and Blair<sup>7</sup> was overlooked during the experimental work reported in the preceding paper. A few analyses have been made in comparing the two methods on pooled samples of swine blood. The results indicate that either method is satisfac-

tory provided that the colorimetric comparisons are made soon after nesslerization. Nesslerized solutions of the Folin-Wu filtrate frequently develop turbidity on standing for fifteen minutes or more. This turbidity does not develop in the nesslerized sodium tungstate sulphosalicylic acid filtrates. From the point of view of simplicity of preparation, the alcoholic urease is more easily prepared and is equally as effective as the concentrated urease in the volume of blood used for analysis.

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## SIMPLIFYING THE MECHANICS OF BACTERIAL FILTRATION\*†

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NUMEROUS articles have appeared in medical and scientific periodicals upon various aspects of filters which remove bacteria from fluids. An extended bibliography is found at the end of a chapter by Mudd in Rivers' volume on Filterable Viruses.<sup>1</sup> The worker who uses filtration as a frequent and important procedure in his work must necessarily familiarize himself with this literature and will gather together many hints and suggestions in regard to the mechanics of the process. On the other hand, the worker who seldom uses filtration usually fails to take time to read so widely and often learns the technic by trial and error. Many times some slight error in the arrangement of the apparatus or some accident in the process of filtering the sample will make it necessary to repeat the operation before a sterile filtrate is obtained. Because of this fact it seemed worth while to put into print a brief discussion of the most frequent pitfalls of the procedure.

Although more convenient types of apparatus for certain purposes have been devised, such as that described by Mudd,<sup>2</sup> the most frequently used arrangement is filtration through a Berkefeld or Mandler candle, or a Seitz disc, into a flask with a side arm, and the suggestions given here will deal with that simple set-up.

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†The laboratory facilities for carrying on an investigation involving filtration are being provided through the courtesy of Dr. W. G. Smillie.

One cause of difficulties often results from the attempt to filter without a manometer to determine the pressure. It is easy to exceed the critical pressure of the candle and draw bacteria through. A manometer is not expensive, and one like that illustrated in Fig. 1 (A) can usually be assembled in a laboratory without having to purchase anything except the mercury and perhaps a piece of tubing with a 1 mm. lumen. The principle of the manom-

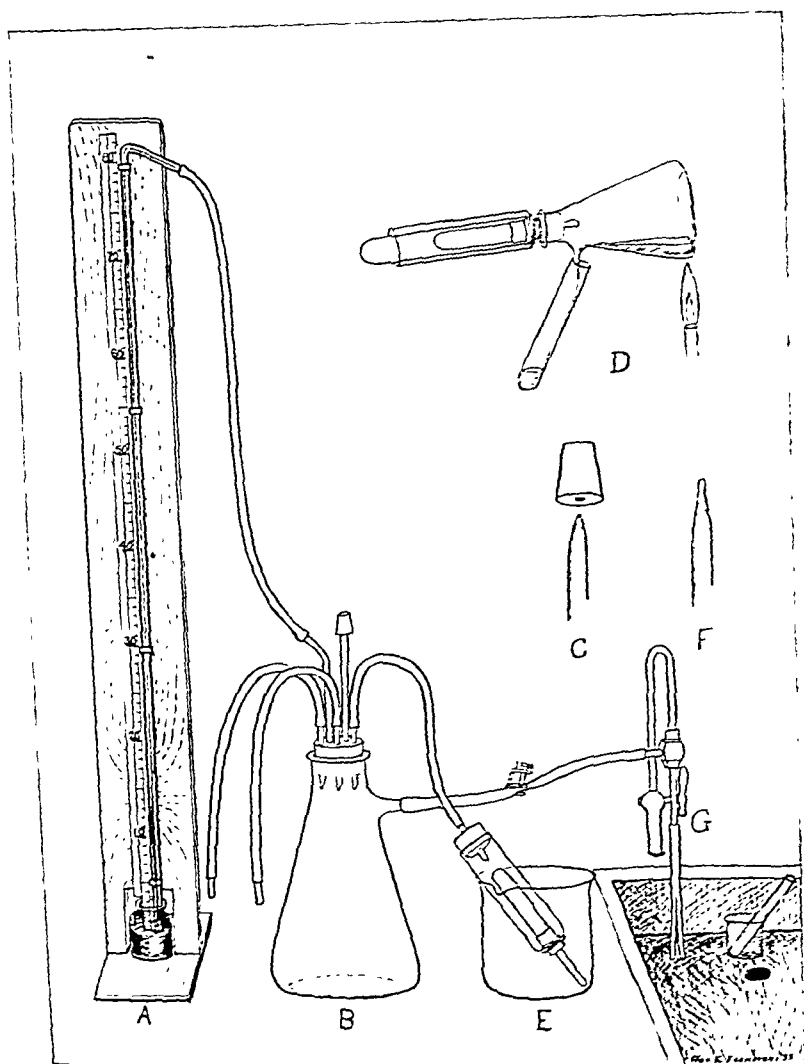


FIG. 1

eter is very simple. It consists essentially of a glass tube standing in a bottle of mercury. Attached to the upper end of the tube is a piece of rubber tubing connected with the flask which is interposed between the water pump and the flask receiving the filtrate. A support for the tube and a metric rule completes the apparatus. The latter should touch the surface of the mercury. It is a good rule to do the filtration at as low a pressure as possible. Most

filtrates can be put through with sufficient rapidity at from 100 to 150 mm. of mercury. When the Berkefeld N filter candles are used, the pressure should never exceed 450 mm. of mercury, and bacteria will sometimes come through at that pressure.

The amount of water going through the vacuum pump should be just sufficient to maintain the pressure at the desired level. A pinchcock on the tubing, Fig. 1 (B), connecting the pump with the filtering flask aids in controlling the pressure and is particularly useful in eliminating the effect of sharp, quick changes in pressure in the water pipes. The orifice left open should be the smallest which will not cause the mercury to drop.

A very annoying thing, which sometimes occurs, is to have the pressure suddenly released when the pump is turned off and to see the cotton plug in the side arm forced into the flask. Refiltration is, of course, necessary since the filtrate is no longer sterile. Such accidents can be avoided by having a valve which will gradually release the pressure. A very simple type of valve is illustrated in Fig. 1 (B) and details are shown in Fig. 1 (C). It consists of a piece of glass tubing which extends through a rubber cork of an intervening flask placed between the water pump and the flask which receives the filtrate. The end of the tube is drawn out and rounded off in a flame until the opening is only about a millimeter in diameter. Over this is pushed a soft rubber stopper in which a hole has been pierced about two-thirds of the distance from the bottom to the top. The tightness of the rubber stopper around the outside of the glass tube prevents the entrance of the air until it is pulled off at the end of the filtration simultaneously with turning off the pump. Air gradually goes in through the small orifice, and the cotton plug in the side arm is not disturbed.

When small quantities are to be filtered, time can be saved and loss due to liquid remaining at the bottom of the mantle can be avoided by inverting a test tube over the filter candle. The tube should be of as small a diameter as possible to still go down to the bottom of the mantle. It should be placed over the candle before the liquid is poured into the mantle. If placed on after the candle is wet, the air is often never entirely exhausted from the inside of the tube, and the fluid level never rises.

Occasionally an accident occurs when taking the rubber stopper from the flask in order to remove the filtrate, particularly if a few drops of fluid happen to fall outside the mantle and down on the stopper. This difficulty can be overcome by removing the filtrate through the side arm, provided care has been taken that no fluid has run down the rubber tube into the side arm and has dampened the cotton. The cotton is removed from the side arm and the end carefully flamed. It is then held over the end of the tube or flask which is to receive the filtrate, as shown in Fig. 1 (D). A flame is applied to the bottom of the flask so that the heating of the air inside causes it to expand and forces the filtrate out. Without the heat the filtrate will not run out of the side arm. When flame is being applied to the side arm, care should be observed not to overheat it, as the liquid will then cause it to crack.



Washing the candles is simple when the arrangement shown in Fig. 1 (*E*) is used. The washing liquid placed in the beaker is pulled through the candle in the reversed direction. The only equipment necessary is a stopper through which a piece of glass tubing passes. The stopper fits snugly into the end of the mantel and the glass tubing is connected with the water pump.

More than one filter can be washed at the same time with the same pump when the arrangement illustrated in Fig. 1 (*B*) is used. Each rubber tube can be connected with a filter, either when filtering or when washing the filters. When only one tube is being used, the other tubes must be closed by a pinch-cock or by thrusting a piece of glass tubing closed at one end, illustrated in Fig. 1 (*F*), into the end of each rubber tube.

Splashing in the sink by the outflow of the water pump can be avoided by the use of a piece of rubber tubing which reaches to one-quarter of an inch of the bottom of the sink. Splitting the lower three or four inches of the tube also reduces the splashing (Fig. 1 *G*).

#### SUMMARY

The various aspects of the mechanics of bacterial filtration are discussed, methods of eliminating the frequent difficulties, which make refiltration necessary, being particularly considered.

Four useful additions to the usual technic are described:

1. A method of using or washing three or more filters simultaneously with the same pump.
2. An easily constructed vacuum release.
3. A simple method of removing the filtrate from the flask.
4. A means of avoiding splashing by the outflow of the water pump.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDEFFE, M.D., ABSTRACT EDITOR

## B. TUBERCULOSIS, Viability and Virulence of Old Cultures of, Corper, H. J., and Cohn, M. L. *Am. Rev. Tuberc.* 28: 856, 1933.

In 1919 and 1920, several hundred 8 ounce nursing bottles containing about 3 ounces of 5 per cent glycerol-broth, neutral to litmus, were planted with 19 different strains of human and bovine tubercle bacilli, and were maintained at 37° C. from then until 1932, when 47 bottles containing cultures of human tubercle bacilli were chosen to determine the viability of the bacilli. Twenty (42.5 per cent) of these were found capable of growing on an inspissated egg yolk medium, while, from 9 bottles planted with bovine tubercle bacilli in 1920, four cultures (44.4 per cent) were viable in 1932 and grew on subculture.

Twelve strains of the original 1919 and 1920 cultures of 17 different strains of human tubercle bacilli subcultured in 1932 grew on the egg medium and were recovered for study and perpetuated, while, of two bovine strains, one grew and was recovered and was perpetuated.

There was noted a certain parallelism between the successful recovery of the bacilli in viable form and the  $P_H$  of the medium at the time of the attempt to grow the bacilli twelve years after planting. None of the transplants grew from bottles in which the acidity of the medium was below  $P_H$  6.1 or the alkalinity above  $P_H$  7.6.

A determination of the approximate number of live bacilli present in the culture masses revealed that about 0.01 per cent of the bacilli in the twelve-year-old culture mass of human tubercle bacilli were still viable, while about 1.0 per cent of the bovine bacilli were still able to grow, conforming to earlier observations by us and other investigators who found bovine tubercle bacilli more resistant to detrimental influences in general than human tubercle bacilli.

It was noted that all of the virulent strains of the twelve-year-old cultures of human tubercle bacilli had not suffered a diminution of virulence during twelve years' residence in the incubator at 37° C., while low-virulent strains remained of low virulence during this time. In contrast, prolonged and repeated artificial cultivation, however, caused a slight but definite diminution of virulence (a slow transition) of human tubercle bacilli in stock cultures. It would appear from this that virulence is a property inherent and unchangeable to individual bacilli but prolonged cultivation on artificial media may cause a perceptible, though not striking, change in the progeny of human tubercle bacilli under the ordinary conditions of laboratory perpetuation on culture media.

These observations also suggest a means for maintaining the original culture of a strain of bacilli as control for tests of methods to enhance or diminish virulence as well as to study other factors of the growth of tubercle bacilli.

The morphologic changes noted in the bacilli in the twelve-year-old cultures conformed to those previously described under the changes of the cytomorphosis of tubercle bacilli (1926).

In correlating these culture findings with *in vivo* conditions of the survival of tubercle bacilli it is evident that the persistence of the bacilli in the body is dependent upon a number of factors, but prominent among these, aside from the pathogenicity of the bacilli, is the factor of the number of tubercle bacilli introduced into the organs and tissues of an animal. This is evident from the fact that while small numbers of virulent tubercle bacilli in fine suspension are removed from the organs and tissues of the rabbit and dog within about one year, large amounts of even avirulent human tubercle bacilli can persist in the tissues of the dog in viable form for over three and a quarter years without the presence of characteristic histologic evidences of tuberculosis in these tissues.

**BLOOD, During the First Year of Life, Merritt, K. K., and Davidson, L. T.** *Am. J. Dis. Child.* 46: 990, 1933.

A group of normal American infants have been studied with reference to the behavior of their erythrocytes, hemoglobin, reticulocytes, and platelets throughout the first year, also the bleeding and coagulation time on the day of birth.

It is apparent that after a period of initial erythrocyte adjustment of two months' duration a mean value of 4.69 million, with a standard deviation of 0.37 million, may be considered as "normal" throughout the first year. After a similar initial period of adjustment, lasting two months, the mean value for hemoglobin for the first year is 12.6 gm. per hundred cubic centimeters, with a standard deviation of 1.36 gm. From these figures, it would appear that normal infants in New York City do not become anemic, as Mackay stated was the case in 51 per cent of artificially fed infants in London. It would seem that her "standard normal hemoglobin curve" is below what may be considered as "normal" in this country. In using Haden's scale of 15.6 gm. per hundred cubic centimeters, which is equivalent to 100 per cent, the color index is found to be 0.86. With Sahli's scale, 17 gm. per hundred cubic centimeters is equivalent to 100 per cent and the color index is therefore 0.90.

The reticulocytes in the normal infant are found to have a mean value of 3.1 per cent at birth. After a rapid drop through the first month, they reach a mean value of 0.16 per cent, with a standard deviation of 0.12 per cent, which is considered normal throughout the first year.

Blood platelets at birth are found to have a mean value of 227 ( $\pm 3.0$ ) thousand. After a rise at the third month, their mean value throughout the remainder of the first year is 344 thousand, with a standard deviation of 66 thousand. The figures at birth are lower than those of other investigators, and rise less rapidly.

Values for the bleeding time at birth are found to be from one-half to two and one-half minutes. The coagulation time at birth is from two to four minutes.

NOTE: Since this article was accepted for publication, Mackey has published an article, "The Normal Hemoglobin Level During the First Year of Life—Revised Figures," in which it was stated that owing to necessary restandardization of her hemoglobin apparatus, it is necessary to add 7 per cent to each value quoted in her monograph (1931).

**LIVER FUNCTION, In Catarrhal Jaundice, Jolliffe, N.** *Am. J. M. Sc.* 186: 640, 1933.

In 19 cases of the type customarily regarded as catarrhal jaundice it has been found that a definite pathologic and physiologic syndrome was present in 16 of the 19. This consists of a regurgitational jaundice which runs through three distinct stages:

a. An "obstructive phase" with an average duration of eleven days in which there is a progressive deepening of the icterus, absent or mere traces of urobilin in the urine, a retention of bromsulphalein paralleling the degree of jaundice and, usually (87.5 per cent), a positive levulose tolerance test.

b. A "critical phase" with an average duration of four days in which urobilin suddenly appears in the urine in high normal or definitely elevated amounts, a coincident abrupt fall in serum bilirubin and bromsulphalein retention.

c. A "recovery phase" with an average duration of nine days during which time the serum bilirubin falls within the level of latent icterus, urobilin is present in the urine in normal amounts and the tolerance to levulose and the retention of bromsulphalein return to normal.

The term "catarrhal jaundice" should be limited to subjects showing this pathologic and physiologic syndrome.

The 3 remaining cases were of distinctly different pathologic and physiologic types. It is suggested that these types may be appropriately designated as acute hepatitis of unknown etiology with, as the case may be, retentional or regurgitational jaundice.

**BLOOD SUGAR, In Schizophrenia, Freeman, W. Am. J. M. Sc. 186: 621, 1933.**

A study is presented of the fasting blood sugar levels as disclosed by 6 samples taken at standard intervals from 59 male schizophrenic patients over a period of six and one-half months.

In 95 per cent of 347 determinations the values lay between the conventional limits of normality, namely, 80 and 120 mg. per 100 c.c. The average value was 96.6 as compared with an average of 95.4 in 31 normal control subjects studied by the same technic. Eight readings were obtained in the hypoglycemic and 8 in the hyperglycemic ranges.

The variation among the schizophrenics was higher than that in the controls, as was shown by the coefficients of variation, 11 and 6.9 per cent, respectively.

The samples were collected in pairs at fifteen-day intervals with intervening periods of two and one-half months. There was a consistent drop in the average blood sugar level from period to period, the first being 99.3 and the third 93.1 mg. per 100 c.c. These findings suggest that habituation with a presumable lessening of emotional reaction to the test played a part in determining the sugar level.

The range of difference in consecutive tests also decreased consistently from one period to another; in the first the average difference was 10.6 and in the last 8 mg. per 100 c.c.

No significant correlation could be recognized between the blood sugar level and the age, period of hospitalization, or severity of the psychosis.

Of the subgroups, the hebephrenics showed the highest average level, namely, 99.7 mg., and the catatonics the lowest, namely 92.7 mg.

**DYSENTERY, Amebic, Unusual Experience With. Ikeda, K. J. A. M. A. 101: 1944, 1933.**

Nine cases of amebic dysentery were recognized within a period of forty-seven days in a general hospital of an average size, in a northern city of 250,000 population, in the United States. This is a unique experience. Infection in all nine cases was definitely traced to Chicago, which demonstrates, probably, a widespread dissemination of this disease throughout the country as a direct result of the Chicago epidemic.

The presenting symptoms show a wide variation, depending on the character of the attack and on the stage of the disease.

The initial symptoms, when elicited, are not always identical. There are atypical cases in which the first symptoms may be misleading or so insignificant and commonplace that no medical relief is considered necessary by the patient.

There is danger of a false positive report in the laboratory diagnosis of this disease by the inexperienced. Careful and painstaking search for the organism should be undertaken in suspected and neglected cases.

Röntgen examination may be of value as an aid in differential diagnosis.

Amebas were demonstrated in a section of a piece of tissue from the rectum, in a case suggesting a malignant growth. The histologic appearance of the lesion presents the characteristic initial changes due to the invasion of *Endamoeba histolytica*.

**TUBERCULOSIS, A Hematological Retrospect, Oatway, W. H., Jr. Am. Rev. Tuberc. 29: 73, 1934.**

The author recapitulates the types of blood picture seen in tuberculosis described by him in 1929 as follows:

1. *Normal*.—The total count and each of the cell-types are within certain normal limits. (Total cells, 6,000 to 9,000; neutrophils, less than 65 per cent, or 5,000 cells; monocytes, less than 10 per cent, or 700 cells; lymphocytes, more than 25 per cent, or 2,000 cells.) It denotes a lack of pathological activity in the lesion, a controlled condition.

2. *Hyperplastic*.—An increase in the mononuclear leucocytes above normal number (more than 9 per cent, or 700 cells). The total count, lymphocytes, and neutrophils are of normal numbers. It denotes new tubercle-formation. A fairly passive type of reaction, both clinically and pathologically.

3. *Septic*.—Primarily an increase of the neutrophiles (more than 65 per cent, or 5,000 cells). There is often an increase of the total count (more than 9,000 cells), and monocytes (more than 9 per cent, or 700 cells), with a lymphopenia (less than 25 per cent, or 2,000 cells). It denotes tuberculous abscess-formation in some degree and is the most serious type of count, associated with all of the more active features of the disease. The level of septicity is, in general, an indicator of the magnitude of the disease process.

A lymphocytosis (more than 32 per cent) added to a normal or hyperplastic count was seen in more favorable conditions (resistant). Single counts were of most value in showing the tuberculous condition only at the time of the count. Repeated counts were recommended to show the trend of the disease. Very few counts were "out of focus" with the actual condition of the patients. A deviation occasionally found was a slightly septic picture in a patient apparently handling the lesion well. Two patients had large lesions and only a hyperplastic blood picture. Such cases were left in the balance for a later judgment.

Previous conclusions as to the meaning and the relative seriousness of the types of leucocyte pictures have been somewhat further reaffirmed by this study of the fate of 250 cases after over three years.

The septic count is the most dangerous type of the blood picture, and is present most often when death occurs, when the illness becomes chronic, or when there is need of surgery. The hyperplastic count is less favorable than the normal and less serious than the septic count. Monoctosis adds to the danger of the septic count when present. The normal count is very good to attain, and a lymphocytosis makes it of even better import.

Compression therapy, especially pneumothorax, has often interrupted the prognosis of an unfavorable count, and in spite of its risks and failures has no doubt modified from poor to good the conditions and counts of many patients. Its use most frequently in cases with septic counts has often modified the course of the disease and the prognosis of the blood picture in this group.

There is nothing in this further survey to suggest that the previous limits or interpretation should be changed or ignored. Although the interpretation of the blood count in a tuberculous individual should be limited to his immediate condition, the fate of 250 patients has been consistent, in general, with the portent of three years or more ago. In spite of this prescient value of one blood count, following the condition of the disease by sedulously repeated counts, care in the interpretation, and a constant adherence to the meaning is the recommended use of the procedure, and it will provide a valuable clinical adjunct in diagnosis of the patient's condition and in surgery.

**ALLERGY: Recent Immunologic Studies in Hypersensitivity to Tobacco, Sulzberger, M. B.**  
*J. A. M. A.* 102: 11, 1934.

The studies reported were carried out, in the main, by means of intradermal tests of the skin with tobacco extracts and with nicotine in cases of thromboangiitis obliterans.

Six concepts of fundamental immunologic nature form the basis of the hypothesis that some cases of certain diseases of the vascular system (thromboangiitis obliterans, angina pectoris and coronary disease), clinically long considered to be in some way connected with smoking, are manifestations of hypersensitivity in certain segments of blood vessels to circulating allergens derived from or contained in tobacco.

Concept 1: There is localized and circumscribed fixed specific hypersensitivity; i.e., a hyper-sensitivity confined to a certain organ or part of an organ may develop.

Concept 2: There is a predilection of certain allergens to sensitize and elicit reactions in certain tissues; i.e., whereas any allergen may sensitize any part, almost all allergens have their favorite point of attack.

Concept 3: Not only sudden and evanescent reactions are elicited when the excitant meets the specifically sensitized shock tissue, but chronic reactions and permanent organic damage are often caused by one or successive shocks resulting from such encounters.

Concept 4: There is an idiosyncrasy, meaning that certain persons react in a manner different from that of the norm; allergens causing reactions in the idiosyncratic person cause

no reactions in normal persons. Allergens are thus usually of harmless nature and are not poisons or toxins in the pharmacologic or toxicologic sense.

Concept 5: The vascular system in man seems to be peculiarly susceptible to sensitizations and is thus most frequently the seat of reactions. Examples of this are found in many diseases of the skin. The wheal reaction is, in itself, a demonstration of vascular hypersensitivity.

Concept 6: The skin is useful as a test tissue in a great variety of hypersensitivities of internal organs.

The experiments in patients with thromboangiitis obliterans and in controls have brought to light the following data: 78 per cent of the patients with thromboangiitis obliterans tested had positive reactions of the skin to tobacco; 36 per cent of the smokers without thromboangiitis obliterans; and 16 per cent of the nonsmokers gave positive reactions.

The persons afflicted with thromboangiitis obliterans were not atopic, and there were no regularly demonstrable reagins in their serums.

As the positive skin tests in patients with thromboangiitis obliterans correspond with the clinical evidence incriminating tobacco, these results are regarded as highly suggestive that sensitization of the vascular system to tobacco may play a causal or contributory rôle in many cases of thromboangiitis obliterans.

Although tobacco was the principal allergen causing positive reactions of the skin in thromboangiitis obliterans, other allergens and, notably, inhalants also elicited positive responses.

It is assumed that cases of thromboangiitis obliterans must exist in which other excitants and not tobacco are the major factors.

That part of the tobacco which elicits the skin reaction of hypersensitivity is coctostable and thermostable and is not destroyed by ultraviolet rays or x-rays in the dosages employed. It is not nicotine.

Nicotine is of little or no importance in sensitizations to tobacco. If the diseases under discussion are sensitizations to tobacco, nicotine is, in all probability, of little or no importance in their causation.

It is hoped that the new immunologic approach and the employment of animal sensitizations will lead to the experimental reproduction of certain diseases clinically attributable to damage by tobacco. As is well known all toxicologic attempts to do this have failed.

One of the objects of such experimental studies would be to isolate and, eventually, to eliminate the actual sensitizing factor or factors from tobacco.

**LIVER FUNCTION, In Advanced Pulmonary Tuberculosis, Steidl, J., and Heise, F. H.**  
*Am. J. M. Sc.* 186: 631, 1933.

In a series of 23 patients having advanced pulmonary tuberculosis all but one gave pathologic readings in one or more of a group of 5 tests for hepatic function. Of the tests used, the cinchophen test, the icterus index and the galactose test were the most sensitive in the order named.

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## EDITORIAL

### The Epidemiology of Amebic Infection

WE HAVE good reason to hope that the great interest in amebiasis, stimulated by the recent Chicago epidemic will be productive of a greatly increased knowledge concerning the epidemiology of this condition. The general understanding based upon reports in the current medical literature is that the Chicago epidemic was the result of food infection following contamination of the food by food handlers, either healthy carriers or individuals mildly ill with amebic colitis. The official report later incriminated defective plumbing systems in the old hotels, in which there was some sort of cross connection between the sewage system and the water supply, such that, with overload, the water supply could become contaminated.

There can be no doubt in view of the reports by Bundesen, of the extremely high incidence of carriers of *Endameba histolytica*, in the hotels chiefly responsible for the epidemic that food contamination played a part, possibly also infected water supply.

The review of the measures for the control of amebic dysentery recently enumerated by McCoy are of great interest in connection with the epidemic. After emphasizing that we do not have sufficient information concerning the factors governing transmission, on which to base authoritative measures, and that the present facts do not justify some of the various measures being put into effect, McCoy summarized present epidemiologic information as follows:

There is very little evidence that the cases originating in Chicago have led to any considerable spread of the infection in other communities into which the infective individuals have gone.

Carriers do not appear as much of a menace as they had been thought to be. There is no clear evidence that carriers, even among food handlers, are important in transmission.

Large scale control of spread by detection of carriers and their removal from food-handling groups appears not practicable.

There is no need for isolation of clinical cases beyond that necessary for the benefit of the patient. There is no need for isolation of carriers.

In the absence of modern methods of sanitary sewage disposal precautions should be taken to prevent water supply contamination and fly contamination. Otherwise no precautions need be taken.

Persons exposed to clinical cases or carriers need no particular attention.

Measures which should be taken by health officers consist in calling the attention of practicing physicians to the importance of recognizing dysentery; insistence upon reporting all cases of dysentery; distinguishing between the amebic and bacillary forms; the provision of facilities for diagnosis; educational measures among food handlers with regard to cleanliness; feces examination of food handlers, measures to prevent possible contamination of drinking water supply, especially in hotels and public eating places.

Spector and Buky report a laboratory investigation in which feces containing ameba histolytica was allowed to dry upon the hand, to determine the viability of cysts. They conclude that the majority of cysts under the circumstances of the experiment were dead at the end of five minutes and that it was very exceptional for any to survive beyond ten minutes.

We infer that the public health service in the discussion just reviewed, are not taking the attitude of the town crier, "twelve o'clock and all's well." Instead, McCoy has presented an analysis of the actual facts as we know them today, with what conclusions are justified by those facts. The reader who does not realize this might be lulled into a false sense of security. The Chicago epidemic was very real, has already caused a very respectable number of deaths, and, as the years go on, will probably be responsible for a great deal of individual illness, the cause of which may remain undetermined. The disease is of sufficient importance, that all promising measures should be taken to prevent its spread, even though some of them may not be completely justified by the facts. Of course one recalls that entire Cuban villages were put to the flame, to destroy yellow fever, which nevertheless returned the next year on schedule. But such useless destruction is not a phase of the prevention of amebic infection.



The clinician is possibly in a better position to study the family incidence of amebiasis than the public health man, and it is this phase of the epidemiology that we would prefer to stress. The reports in the literature together with the experience of all clinicians who see any amount of amebic infection, offer convincing evidence of contact transmission in the family. It seems to us that for the present at least every possible precaution should be taken to recognize amebic infection among food handlers, both in public eating places and in the home, and to prevent this source of food contamination.

Craig, Kofoed and others have for years been preaching the wide distribution of this malady. To an extent they have been crying in the wilderness, but the Chicago epidemic has fully justified their contentions.

Craig emphasizes that, although infection with *ameba histolytica* is more prevalent in the South than in the North, scarcely a state in the country is free from it, and a conservative estimate indicates that between 5 and 10 per cent of the population of the country, as a whole, harbor the parasite. The majority of infected individuals in this country do not develop frank amebic dysentery, as they do in the tropics.

He states that the cysts are the infecting agents. Under normal conditions the motile trophozoites are destroyed by the gastric juice. Persons suffering with acute amebic dysentery do not have cysts in the stools. They are, therefore, of little importance in contagion. It is the individual with few symptoms and the healthy carrier, who has cysts, who are according to Craig the more important epidemiologic consideration. Acute cases untreated or improperly treated, become carriers of cysts.

He finds that transmission through contaminated water supply is common in localities without modern supply and plumbing systems, particularly when wells, springs, and tanks are depended upon for water. The cysts may live for days and even weeks in water, depending upon the temperature and the number of bacteria present. This method of transmission is of importance in this country only in rural districts. "Wherever sanitation and personal hygiene is poor, amebiasis is very prevalent, whether in the tropics, subtropics, or the temperate zones."

Human excreta used for fertilizer is more of a factor in the Orient than in this country. The cysts will remain viable in moist feces for as long as two weeks. Craig also believes that fly contamination is a distinct factor.

"The transmission of the infection by food handlers is practically a certainty unless the greatest care is taken regarding personal hygiene and the cleanliness of the hands of all who handle food. The incidence of infection in food handlers employed in public eating places is often very high, due to their close association and the constant intake of the cysts in food which they themselves contaminate, and one infected food handler may infect many others with whom he is associated, until the vast majority of the food handlers in a particular institution may show infection."

Craig also emphasizes that there has been no convincing evidence of variation in virulence with different strains of *Endameba histolytica*. Walker and

Sellards successfully produced acute amebic dysentery in human volunteers, with cysts obtained from symptomless carriers.

During the next few years it is obligatory upon all clinicians and clinical laboratory workers to emphasize and reemphasize the importance of careful study of every patient with indefinite or unidentified gastrointestinal symptoms, particularly colonic symptoms, with the possibility of chronic low grade amebic infection in mind. Fortunately, two reliable methods are available, direct examination for motile ameba and for cysts, and the complement fixation reaction which has been developed by Craig.

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—W. T. V.

# *The Journal of Laboratory and Clinical Medicine*

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No. 8

## *CLINICAL AND EXPERIMENTAL*

### THE ETIOLOGY OF GRANULOPENIA (AGRANULOCYTOSIS)\*

WITH PARTICULAR REFERENCE TO THE DRUGS CONTAINING THE  
BENZENE RING

ROY R. KRACKE, B.S., M.D., AND FRANCIS P. PARKER, B.S., M.D.  
EMORY UNIVERSITY, GA.

THE disease known as agranulocytosis was described as a clinical entity only eleven years ago.<sup>1</sup> The first case in the United States was reported nine years ago.<sup>2</sup> Since that time it has been constantly on the increase and at this time we have collected 473 cases from the American and Canadian literature alone. It is apparently increasing in frequency and also being recognized more readily.

In our review of the American cases we have been impressed by the consistently increasing number of the acute fulminant types and also by the large number of so-called chronic granulopenic patients as reported by Roberts and Kracke.<sup>3</sup>

It is quite probable that the so-called acute granulopenia in which the granulocytes entirely disappear from the peripheral blood represents only a fraction of the total number of individuals whose blood count is depressed to some degree. Although there was a sporadic case of this disease prior to 1922, it certainly must have occurred very infrequently.

Pepper<sup>4</sup> believes that it was quite common many years ago and cites the numerous instances of so-called putrid sore throat or malignant angina. But

\*From the Department of Pathology, Emory University School of Medicine.

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Read in Section on Pathology, Southern Medical Association, Twenty-Seventh Annual Meeting, Richmond, Virginia, November 14 to 17, 1933.

this assumption is entirely unwarranted since the single diagnostic criterion, namely, a low leucocyte count, was lacking in these cases. The disease is one that is easily diagnosed, runs a dramatic and clear-cut clinical course, and the fact that it was not observed before 1922 is practical assurance that it did not exist before that time. To state otherwise would be to reflect on the diagnostic acumen of our clinicians of the past. Blood counts have been taken for fifty years. We must consider, then, that granulopenia is a disease of modern times and one that has come among us during the past ten years.

#### ETIOLOGY

*Live Bacteria.*—Since the first report of Schultz<sup>1</sup> in Germany and the report of Lovett<sup>2</sup> in the United States, many investigators have directed their efforts toward the solution of the etiologic factors. Up to this time these efforts have met with very little or no success. It is the chief purpose of this paper to present briefly a summation of the work in this field and to formulate an hypothesis for the cause of granulopenia and to present evidence supporting this view.

Lovett<sup>2</sup> noted in her case the presence of mouth ulcers infected with *B. pyocyaneus* and at once suspected this organism as etiologic. She injected numerous laboratory animals but failed to produce the condition.

Similar observations have been made by Linthicum,<sup>3</sup> Windham,<sup>4</sup> Keeney,<sup>5</sup> Also, Friedmann<sup>6</sup> in a study of 23 cases found *B. pyocyaneus* in the blood stream of one. It will be noted that this same organism has been found in the blood stream of three of the American cases (Table I). These findings have led many to suspect it as etiologic but up to this time efforts to reproduce the disease in laboratory animals with it, have been unsuccessful. There is little support for the bacterial etiology of granulopenia since it has been well demonstrated by Roberts and Kracke<sup>7</sup> that the basic pathology is first, the disappearance of the neutrophils, and this in turn is followed by the invasion by any and every organism that is accessible, particularly throughout the entire length of the gastrointestinal tract. Most writers are in accord with the conception that granulopenia is first a disease of the bone marrow, followed by disappearance of peripheral granulocytes, this in turn followed by varying degrees and types of infectious processes.

It will be noted from a study of Table I that at least 25 organisms have been found as blood stream invaders in this disease and that approximately 20 per cent of the cases show blood stream infection. The wide variety of invading bacteria can also be noted from the numerous types that are responsible for the ulcers. A few reports tending to incriminate various organisms have been made. Notable among these is the work of Fried and Dameshek<sup>8a</sup> in which they reported the production of primary granulopenia in rabbits by the intravenous injection of *Salmonella Suipestifer*. An analysis of their report shows that in no instance did they reproduce the condition as it is observed clinically, since they failed to produce a sustained and prolonged depression of the leucocyte count, but did produce a temporary neutropenia which, as has been well demonstrated, will follow the injection of any killed organisms or other matter in a finely divided state.

TABLE I  
 BLOOD STREAM INFECTION IN GRANULOPENIA

| ORGANISM                                 | NUMBER OF CASES |
|------------------------------------------|-----------------|
| <i>Streptococcus hemolyticus</i>         | 8               |
| <i>Streptococcus viridans</i>            | 15              |
| <i>Streptococcus</i> (type undetermined) | 9               |
| <i>Staphylococcus albus</i>              | 3               |
| <i>Staphylococcus aureus</i>             | 4               |
| <i>Bacillus Friedlander</i>              | 4               |
| <i>Pneumococcus</i> , Type 2             | 3               |
| <i>Pneumococcus</i> , Type 3             | 1               |
| <i>Pneumococcus</i> , Type 4             | 2               |
| <i>Pneumococcus</i> , Type undetermined  | 2               |
| <i>Diplococcus</i> (undetermined)        | 4               |
| <i>Bacillus pyocyaneus</i>               | 3               |
| <i>Bacillus paratyphosus</i> B.          | 1               |
| <i>Bacillus coli</i>                     | 3               |
| <i>Bacillus proteus</i>                  | 1               |
| <i>Streptothrix</i>                      | 3               |
| <i>Streptococcus</i> with other bacteria | 9               |
| <i>Fusiform bacillus</i> (Gram negative) | 1               |
| Estivo-autumnal parasites                | 1               |
| <i>Varines cocci</i>                     | 3               |
| <i>Staphylococcus</i> (undetermined)     | 1               |
| <i>Diphtheria bacillus</i>               | 1               |
| Vincent's organisms                      | 1               |
| Typhoid bacillus                         | 1               |
| <i>Bacillus</i> (undetermined)           | 1               |
| Total number of positive cultures        | 74              |
| Negative, or not stated                  | 395             |

1. Blood cultures are positive in about 20 per cent of the total number of cases or positive in 70 per cent of the examined cases.

2. The streptococci are the most frequent invaders.

3. The blood stream infection is probably secondary to the leucopenic state.

The clinical onset of many cases of granulopenia has been preceded by tooth extractions, treatment for pyorrhea, mouth ulcers, etc. It will be noted in a study of Table II that this is a common history and in many of these cases Vincent's organisms have been isolated. We believe that these oral

 TABLE II  
 IMPORTANT FEATURES OF THE HISTORIES

|                                                      |    |
|------------------------------------------------------|----|
| Tooth extraction                                     | 27 |
| Treatment for pyorrhea, etc.                         | 22 |
| Treatment for syphilis                               | 22 |
| Influenza (mild)                                     | 17 |
| Tonsillectomy                                        | 5  |
| Sore throat                                          | 85 |
| Acute coryza                                         | 15 |
| Malaise and weakness                                 | 91 |
| Sudden onset (no premonitory symptoms)               | 91 |
| Various operations                                   | 48 |
| Abscesses and furunculosis                           | 8  |
| Arthritis                                            | 7  |
| 93 other clinical conditions, 1 to 2 in each patient | 6  |
| Not stated adequately                                | 77 |

1. Whether or not the numerous instances of dental therapy preceding the disease are the results of a previously unrecognized leucopenia has not been settled.

2. Nearly three-fourths of all reported cases had been receiving treatment for some other disease for variable periods prior to their attacks of granulopenia.

3. It is interesting that eight patients developed the disease while in a hospital for treatment for other conditions.

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2. Nearly three-fourths of all reported cases had been receiving treatment for some other disease for variable periods prior to their attacks of granulopenia.

3. It is interesting that eight patients developed the disease while in a hospital for treatment for other conditions.

manifestations are secondary to a preceding neutropenic state. It must be borne in mind that when the granulopenic patient consults a physician he presents the terminal stage of a condition that has existed possibly for many months. And, furthermore, many people have developed acute fulminant granulopenia and have died without showing any ulcers whatever. There has been no experimental or clinical evidence to indicate that Vincent's organisms are capable of producing a severe and sustained leucopenic state.

There are many reports in the literature concerning the efforts of various investigators to reproduce the disease in laboratory animals with organisms that have been isolated from their respective patients, but all of this work has been unsuccessful. Piersol and Steinfeld<sup>10</sup> injected intravenously into rabbits inactivated cultures, Berkefeld filtrates, and supernatant fluids of cultures of many organisms and failed to reproduce the condition and called attention to the fact that a temporary leucopenia may be produced by the injection of peptones and a large number of other proteins.

It has been stated that granulopenia may be due to the continued absorption of toxins from sites of focal infection. The recent work of Dennis<sup>11</sup> is important. He was able to produce a sustained and marked leucopenia in rabbits in which he allowed the *Streptococcus viridans* to grow in the tissue under such conditions that the toxins were diffused throughout the animal body while the organisms remained in situ. His results were not conclusive in the use of *Staphylococcus aureus*, *Streptococcus hemolyticus*, and *B. proteus*. It will be noted from a study of Table I that *Streptococcus viridans* has been reported as the most frequent blood stream invader in this disease. Dennis<sup>12</sup> stated later that a weakness of his experimental work consisted in the use of a very large dose in relation to the size of the animal. It has long been known, of course, that overwhelming infection with certain organisms will produce variable degrees of leucopenia.

*Dead Bacteria.*—The injection with dead bacteria in the course of vaccination has been suggested as a possible cause. Bromberg and Murphy<sup>13</sup> reported a typical case following a series of typhoid vaccine injections. Kracke<sup>14</sup> has reported a similar case in which, however, the patient had taken so much phenacetine during the previous four-year period that she was suffering from a severe sulphhemoglobinemia. Dr. R. P. Irwin<sup>15</sup> of Moulton, Ala., has submitted a record of a typical case following typhoid vaccination. In his case, also, the patient was under treatment for various other conditions in which she was given considerable medication of various types. Pepper<sup>4</sup> has advanced the conception that the disease may be allergic in nature, but there is little evidence to support his view.

*Glandular Dysfunction.*—The influence of the endocrine glands on the output of both granulocytes and erythrocytes has been studied considerably and has long been suspected.

Hubble<sup>16</sup> summarizes this work and postulates that bone marrow depression may be caused by a pituitary basophilic insufficiency, and he states that there is much evidence to indicate that granulopenia may be caused by cortical adrenal dysfunction.



Britton and Corey<sup>17</sup> have reported that experimental adrenal insufficiency in cats resulted in a marked degree of neutropenia with the return of the granulocytes to normal following administration of corticoadrenal extract. They did not produce, however, the typical granulopenic syndrome as is commonly seen.

Jaffe<sup>15</sup> has studied a patient with generalized infiltration of lymphadenoma which included invasion and destruction of the adrenal cortex with the patient finally dying with a typical granulopenia. He infers from this that the granulopenia was due to a cortical adrenal insufficiency.

Kunde et al.<sup>19</sup> have noted the production of a hypoplastic and aplastic bone marrow in thyroidectomized rabbits. Evidence accumulated indicates that the ductless glands in their various relationships have an effect on the output of blood cellular elements. This conception, however, will have to await further confirmation.

*Radiation.*—Excessive exposure to radiation has been suspected as a possible etiologic factor. It is known that Thorium X will produce a marked neutropenia in animals and in the human being. It is conceivable, of course, that excessive roentgen radiation can produce marked bone marrow depressions, and it should be used with caution in the therapy of this disease. Also, it will be noted from a study of Table III that granulopenia is a disease essentially of the white race, being quite rare in negroes, and this may have some relationship to the radiation theory.

*Bone Marrow Idiosyncrasy.*—This rather ill-defined conception includes the opinions of a considerable number of writers all of whom postulate that an occasional individual has a weakened or faulty bone marrow which will become nonfunctional if submitted to undue strain or stimulation.

Beck,<sup>20</sup> in her excellent review of this disease, holds the view that the primary pathology will not be found in the marrow itself, but in that particular organ or tissue which regulates granulopoiesis. She believes that those cases showing peripheral neutropenia but with a normal bone marrow would

TABLE III  
THE INCIDENCE OF GRANULOPENIA

|                                      |     |
|--------------------------------------|-----|
| Number of cases in the United States | 473 |
| Number of cases in the white race    | 442 |
| Number of cases in the colored race  | 8   |
| Race not stated                      | 23  |
| Number of cases in males             | 161 |
| Number of cases in females           | 310 |
| Sex not stated                       | 2   |

1. The disease is essentially one of the white race.

2. It exists in the ratio of two females to one male.

be due to a lack of the chemotactic factor, whereas the damaged bone marrow would be due to a lack of the maturation factor. As Beck infers, it is true that the bone marrow in some cases, both at autopsy and sternal biopsy, shows a normal or hyperplastic state while other cases show a definite aplasia of the marrow.

Fitzhugh and Krumbhaar<sup>21</sup> are of the opinion that the bone marrow may be hyperplastic up to the myelocytic level but that maturation ceases at that point.

Kracke<sup>22</sup> has studied a case which showed a definite hyperplastic bone marrow at sternal puncture at a time when the peripheral cell count was 500. This was explained four days later when the count reached 25,000. Finally this patient died with a second attack, showing a markedly aplastic bone marrow at autopsy.

If Beck's conception is correct, it follows that there is sometimes a temporary restriction of either the maturation or the chemotactic factor. Her theory, however, does not explain why such derangement occurs.

*Chemicals.*—It has long been known that various chemicals will depress the marrow function, resulting in complete inhibition of all cellular types. For example, benzene poisoning has been recorded as a process in which hematopoiesis is completely inhibited in all of the three bone marrow elements, resulting in a picture simulating aplastic anemia. It has been shown by Kracke<sup>23</sup> that benzene may be introduced into the rabbit in such small doses that it exerts a selective affinity for the granulopoietic tissue only, leaving the erythrocytic and thrombocytic elements relatively undisturbed. This same situation is conceivably true with other bone marrow depressing agents, and, no doubt, is true in the case of arsphenamine. Arsphenamine poisoning is a known etiologic agent in the production of bone marrow depression. The clinical picture resulting has been chiefly one of anemia, associated with granulopenia and thrombocytopenia as well. It will be noted from a study of Table II that at least 22 of the cases of granulopenia have been preceded by arsphenamine therapy, and there is little doubt that the drug was etiologic in that particular group of cases. But still more important, it shows that arsphenamine is capable of producing a type of bone marrow depression restricted chiefly to granulopoiesis to such an extent that the clinical syndrome resulting cannot be differentiated from true idiopathic granulopenia.

It has been shown that the injection of the human being with certain preparations of gold will produce a condition closely simulating, if not identical, with true granulopenia. Many such cases have been reported from France, Achard et al.,<sup>24</sup> Ameuille and Braillon,<sup>25</sup> Angeras and Ginsbourg.<sup>26</sup>

The fact that typical granulopenia can be produced with gold preparations and with arsphenamine preparations which contain the benzene ring is evidence that this class of products can, and in some instances does, manifest a selective action on the granulopoietic tissue only. It seems, therefore, in the study of etiology that it becomes our problem to determine which of these chemicals has come into wider usage and closer contact with our people during the last ten-year period or during the period of agranulocytosis.

#### THE INCIDENCE OF GRANULOPENIA

Our review of the reported cases in the United States and Canada has uncovered many interesting findings in connection with the occurrence of this disease.

*Geographic Distribution.*—We have collected from the literature reports of 473 cases from 1922 to 1932 inclusive. There has been an increasing number reported each year, probably because of the larger number of cases, and its more widespread recognition. During this same period there have been reported in Germany approximately 350 cases. The French literature reveals about 100 cases in that country. The disease is apparently quite rare in Russia, Poland, and the Far East, and, for some unknown reason, it is extremely rare in Great Britain. It may be more prevalent in the Far East than we suspect, but the cases probably are not reported.

Dennis<sup>12</sup> has observed two cases in the University Hospital at Beirut, Syria, and states that he discussed the incidence of the disease with several physicians from Palestine and Egypt, and practically all of them had seen cases. Dr. Kleeberg who is practicing in Jerusalem said he had observed the condition there. Up to 1929 there had been only three cases reported from Russia. The rarity of the disease in England is of special significance. It is quite evident that it does not occur in that country to any appreciable extent. As we shall attempt to show, this may have its cause in the type of drugs that are employed by physicians of Great Britain. Briefly stated, granulopenia occurs mainly in the United States and Germany (see Table IV).

TABLE IV  
GEOGRAPHIC DISTRIBUTION OF GRANULOPENIA (FROM 1922 TO 1932)

|               | NUMBER OF CASES |
|---------------|-----------------|
| United States | 473             |
| Germany       | 350 (Approx.)   |
| France        | 100 (Approx.)   |
| England       | 6 (Total)       |
| Italy         | 50 (Approx.)    |

1. The disease is extremely prevalent in Germany and the United States.

2. It is extremely rare in England.

*Age, Sex, and Race.*—A study of Table III reveals the highly significant finding that granulopenia is a disease primarily of the white race, since only eight reports of its occurrence in the colored race were found, and in the

TABLE V  
THE SEASONAL INCIDENCE

| MONTH      | NUMBER OF CASES |
|------------|-----------------|
| January    | 32              |
| February   | 27              |
| March      | 39              |
| April      | 29              |
| May        | 26              |
| June       | 32              |
| July       | 23              |
| August     | 26              |
| September  | 17              |
| October    | 25              |
| November   | 23              |
| December   | 26              |
| Not stated | 159             |

1. There is no apparent seasonal variation.

majority of these, the onset was preceded by arsphenamine therapy. It occurs in the ratio of two females to one male, and this is also true of the cases from other countries. It has no particular seasonal incidence as shown by Table V as cases occur in about equal number throughout the months of the entire year.

The average age is between forty and fifty and ranges from under one year of age to above eighty. It is, therefore, a disease chiefly of middle life.

*Occupation.*—In our studies we have paid particular attention to the occupation of the reported cases and this study has revealed some very interesting and valuable information. It is a striking fact that granulopenia is more prevalent among physicians and their relatives, nurses, hospital employees, and medical students than in any other group of people. Based on the 1930 population census of the United States, it occurs fifty times more frequently in physicians than in lawyers, and two hundred times more frequently in nurses than in female school-teachers. We have made this observation before and have called attention to this singular finding.

It will be noted that of the 200 reported cases in which the occupation is stated, including the housewives, approximately 10 per cent of these have been physicians and members of the medical group. The so-called medical group, including physicians, nurses, hospital employees, etc., constitute 60 per cent of the reported cases of agranulocytosis in the United States in that group in which the occupation is definitely stated. It is not justified to explain this on the belief that this illness is diagnosed more promptly or efficiently in this group of people, since it is well known that physicians are most negligent concerning their own health.

This observation has been noted by several writers. In 1931 Stelhorn and Amolsch<sup>27</sup> in summarizing 42 cases of granulopenia from the region about Detroit, Michigan, made this significant statement, "It is rather curious to note that many of these patients were members of the medical or allied professions, or were relatives of physicians." In their series of cases were 2 nurses, 2 physicians, 1 dentist, and 7 immediate relatives of physicians. Studies of the reports of large series of cases show this same finding to be invariably true. In Harkin's<sup>28</sup> group of 8 cases there was one doctor, one medical student, and one nurse. In a series of about 15 patients from the Mayo Clinic<sup>29</sup> the 2 cases of typical acute fulminant granulopenia occurred in a doctor and a nurse. Hinton<sup>30</sup> and his associates in Macon, Georgia, have studied 6 cases in that section, of which one was a physician and one was a nurse. Sydenstricker<sup>31</sup> reported one case from Augusta, Georgia, who was a physician. Madison and Squier<sup>32</sup> have recently reported from Milwaukee, Wisconsin, a valuable observation, in which they studied 14 patients, of whom 5 were physicians, physicians' wives and nurses, and they stated, "In this group as in the disease at large, there seems to be a most remarkable relation to the medical and allied professions." Fitzhugh and Comroe<sup>33</sup> reported 18 cases from the University of Pennsylvania, and one of them is listed as a physician. Kracke<sup>34</sup> has studied 11 cases around Atlanta, Georgia, in which

there was one physician, one nurse, and one dentist's wife. It is interesting that the only case report we have noted from Poland was one of a physician. Furthermore, we have been advised<sup>12</sup> that 2 physicians are included in the small group of cases around Denver, Colorado.

These observations leave little doubt but that granulopenia is a disease which is peculiar in its relation to the medical profession. Our chief problem then is to determine the possible etiologic factors that could enter into the lives of people in the medical and allied professions that would produce such a large number of cases in this group (see Table VI).

TABLE VI  
STUDIES ON OCCUPATION

|                         |        |
|-------------------------|--------|
|                         | 102    |
| Housewives              | 22     |
| Physicians              | 17     |
| Nurses                  | 4      |
| Maids in hospital       | 3      |
| Medical students        | 1      |
| Laboratory technician   | 11     |
| Relatives of physicians | 2      |
| School-teachers         | 5      |
| Farmers                 | 10     |
| Business men            | 2      |
| Clerks                  | 3      |
| Sailors                 | 1      |
| Dentist                 | 1 each |
| 49 other occupations    | 289    |
| Occupation not stated   |        |

1. The disease is extremely prevalent in physicians and their relatives, nurses, and hospital employees.

2. It is fifty times more prevalent in physicians than in lawyers.

3. It is two hundred times more prevalent in nurses than in female school-teachers.

(Based on population from U. S. Census.)

*Class of People.*—Kracke<sup>14, 23, 34, 35</sup> has repeatedly called attention to the fact that this disease occurs in the better class of our people. In 11 cases in the Atlanta section were included the following: (1) A retired, elderly, cultured woman; (2) a retired, elderly, wealthy woman; (3) a wife of a leading business man in a small town in Georgia; (4) a chief of police in a Georgia city; (5) a wife of an Atlanta dentist; (6) chief librarian in a girls' college; (7) a cultured, refined widow who was economically, moderately well-to-do; (8) the wife of a leading Atlanta architect; (9) a practicing physician; (10) a nurse in the University hospital; (11) a middle-aged white man in the municipal hospital of Atlanta (who was a patient for two months there before developing the disease).

In this connection we quote from the report of Stelhorn and Amolsch<sup>27</sup> as follows: "Very few of the cases occurred among the poor classes. Receiving Hospital, a large hospital maintained by the City of Detroit for the poor, and having an excellent staff of alert, well-read physicians, has no record of any cases of primary granulocytopenia. At least 9 of 31 true cases are extremely well-to-do. Locally the disease is most common among the people of the better classes."

To summarize the peculiar distribution of granulopenia based on our present observations and an exhaustive review of the literature, we would

state: that it is a disease which definitely occurs in the better class of our people; that it has a peculiar predilection for members of the medical and allied professions; that it is confined almost exclusively to the white race; and that it occurs chiefly in Germany and the United States.

#### THE RELATION OF DRUG THERAPY TO GRANULOPENIA

We have long felt that the peculiar distribution of granulopenia could well be correlated with the use of certain benzene containing drugs that are commonly used for therapeutic purposes. We called attention to this in June, 1930, at which time we believed that the benzene ring was incriminated in the production of this disease. At that time we reported our efforts to produce experimentally granulopenia in rabbits using some of the various oxidation products of benzene and using, also, a series of drugs which contained the benzene ring as their nucleus. We have investigated as carefully as possible the various etiologic factors in this respect in eleven cases of true idiopathic granulopenia with the following findings:

CASE 1.—An elderly white woman who had been subjected to repeated cystoscopic examinations over a period of nearly a year, during which time she was given frequently a large number of Peralga tablets (which contain 71 per cent of amidopyrine).

CASE 2.—A middle-aged white woman in whom the onset was preceded by a protracted period of illness diagnosed as influenza, during which time she was given practically daily amidopyrine and empirin compound for a period of several weeks; and prior to that time she had taken about three tablets two or three times a month over the past four-year period.

CASE 3.—A middle-aged white man had taken for more than a year, as much as 15 gr. of amidopyrine daily for attacks of precordial pain, interspersed with a prescription containing phenacetine. Just before the clinical onset he was treated for a condition diagnosed as a mild attack of influenza and was given a prescription of capsules containing phenacetine.

CASE 4.—A middle-aged white woman, the wife of a dentist, had taken a proprietary preparation known as James' Tablets several times weekly over a period of from five to ten years, according to her husband. He stated that his wife took these tablets for every ache and pain. Investigation showed that each tablet contained one grain of acetanilid.

CASE 5.—A young white woman, a nurse in the Emory University Hospital, had taken from 5 to 15 gr. of amidopyrine daily during her menstrual periods for a period of several years and had taken them more frequently during the seven or eight weeks preceding the onset of her illness.

CASE 6.—A middle-aged white woman, librarian, had emotional stress during her last year of life. She was known to have suffered from profuse menstruation and, according to such information as was available, was thought to have been addicted to the use of the so-called coal tar drugs.

CASE 7.—A middle-aged white woman had received three doses of neoarsphenamine and had taken empirin compound for her various minor symptoms (phenacetine, aspirin and caffeine).

CASE 8.—An elderly white woman gave a history of prolonged use of compounds containing phenacetine which had been prescribed for her by her dentist in connection with a long period of dental work.

CASE 9.—A middle-aged white woman had been given phenacetine for five years preceding the onset of her illness, and during this five-year period had taken so much that when she was admitted to the hospital with granulopenia, she presented a typical picture of phenacetine poisoning with the marked slate blue color of sulphhemoglobinemia.

CASE 10.—A middle-aged physician in general practice was known by his brother, who was a dentist, to be a coal tar drug addict, to such an extent, that his brother had even at

times remonstrated that he took entirely too many of these drugs. His brother stated that he had taken such drugs as empirin compound, allonal, pyramidon, etc., almost daily for years for the slightest ache or pain.

CASE 11.—A middle-aged white man admitted to Grady Hospital (Municipal) Atlanta, for treatment of rectal fistula. He was in the hospital for two months during which time his medication covered a wide range of drugs. Included in this were generous doses of pyramidon. It is not known to what extent he took these preparations before his admission to the hospital. It is noteworthy that this patient developed granulopenia only after he had been in the hospital for two months and is the only case of granulopenia that has been observed in the 600 bed Municipal Hospital of Atlanta.

The cases outlined above were of the true idiopathic granulopenic type and do not include the chronic forms.

We have felt for the past three years that members of the medical profession have been prone to prescribe and use this class of drugs for their own ailments and for members of their families. We also feel that the average hospital nurse in the course of her duties, when seeking a mild analgesic, will, because of her familiarity with these newer preparations, employ one of the coal tar drugs instead of the older remedies such as aspirin. We feel also, that average laymen when treating their own aches and pains will use aspirin unless they utilize some drug that has been prescribed for them by their physicians. We have further observed that the average negro who, incidentally, is not subject to as many minor complaints and illnesses as the white person, knows little about this class of drugs and seldom, if ever, uses them on his own initiative.

In order to verify these points, we have conducted a study of several of these groups with particular reference to the usage of the coal tar drugs. This information was obtained by history blanks which were filled out by the following groups of people: (1) 154 student and graduate nurses; (2) 100 physicians; (3) 100 random admissions to the University Hospital (white); (4) 100 admissions to the Municipal Hospital (colored). The results obtained from this series of questionnaires will be seen in Table VII.

To summarize the information from this table, it will be noted that of those nurses who resort to the use of drugs, 76 per cent will select a drug containing the benzamine group; second, of those physicians who will prescribe for themselves and their own families, 56 per cent will use drugs of the benzamine group; third, of the average white lay patients, approximately 27 per cent will select drugs of this class; and, in the colored group about 15 per cent would use this type of drug.

TABLE VII

|                                           | NURSES | DOCTORS | LAITY | NEGROES |
|-------------------------------------------|--------|---------|-------|---------|
| Total number of histories                 | 154.0  | 100     | 100.0 | 100     |
| Total number using drugs                  | 124.0  | 100     | 65.0  | 22      |
| Number using benzamine drugs              | 94.0   | 55      | 17.0  | 15      |
| Percentage using benzamine drugs          | 75.8   | 55      | 26.1  | 15      |
| Number using acetylsalicylic acid         | 26.0   | 45      | 44.0  | 7       |
| Percentage using acetylsalicylic acid     | 20.8   | 45      | 67.9  | 7       |
| Number using drugs not classified above   | 4.0    | --      | 4.0   | --      |
| Per cent using drugs not classified above | 3.3    | --      | 6.0   | --      |

It can be seen, therefore, that there exists almost a direct proportion between the incidence of granulopenia in these groups and the incidence of drug

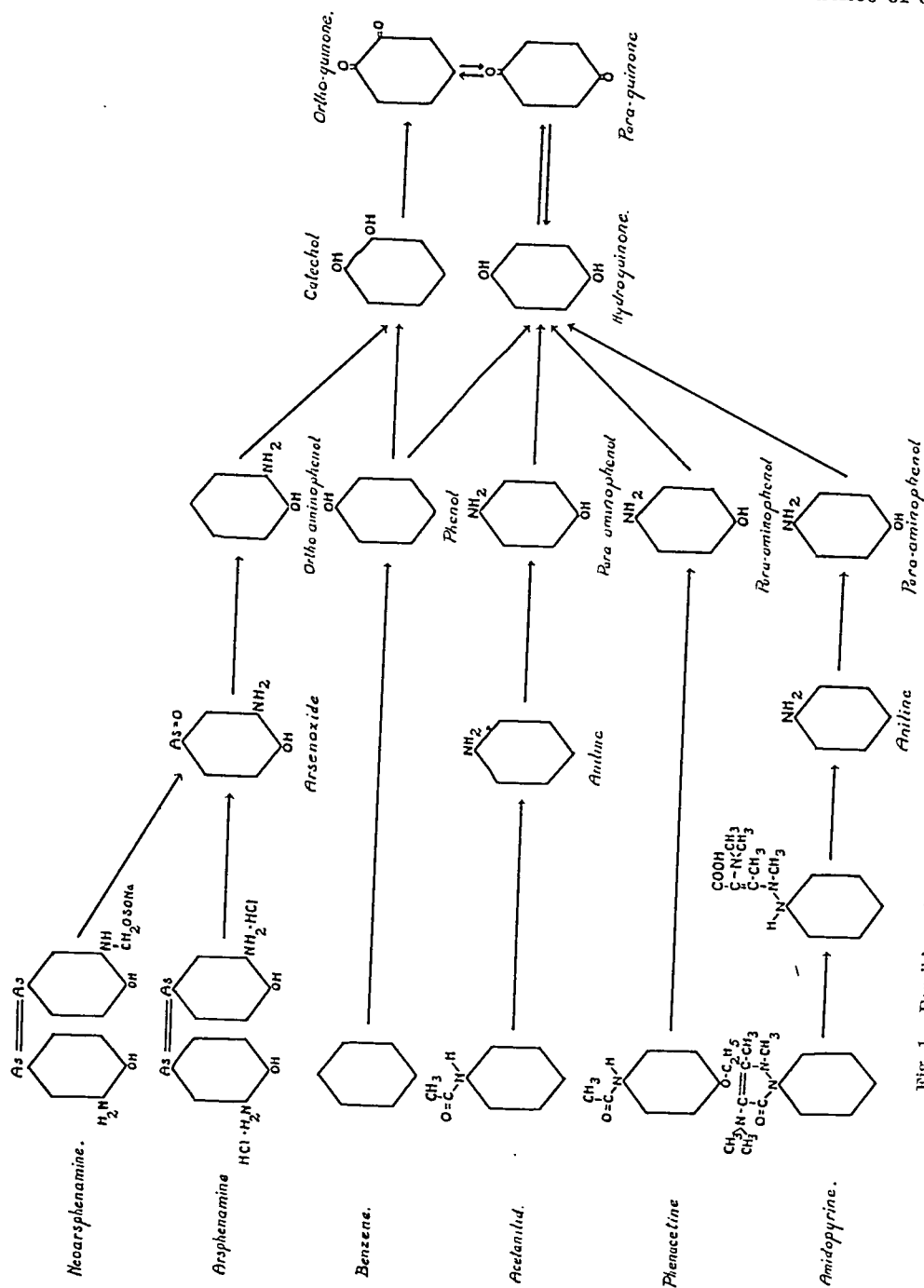


Fig. 1.—Possible oxidation reactions of the benzamine group of drugs and of benzene.

usage in the same groups. In other words, if a nurse needs an analgesic 3 out of 4 will select a coal tar drug and 3 physicians out of 5 will use the same class of drug, whereas, only 1 white layman out of 4 and 1 negro out of 8



will use the benzamine drugs. We believe, therefore, that there exists a direct relationship between the usage of these drugs to the incidence of granulopenia in these various groups.

The peculiar geographic distribution of granulopenia can also be correlated with usage of this class of drugs. The prevalence of the disease in Germany and the United States approximates the use of coal tar derivatives of this class in these two countries. The practical absence of the disease in England is correlated with the fact that this class of drugs is used to a lesser extent in that country. It is also known that some of these drugs, notably those in combination with barbiturates, have been introduced only within the last ten- or twelve-year period which is the time period of granulopenia. Therefore, based upon these statistical studies and upon our observations in a series of 11 cases of granulopenia and on our studies of drug usage in these various groups of people, we believe that this must be seriously considered as an etiologic factor in this disease.

In October, 1933, Madison and Squier<sup>22</sup> presented a report of 14 cases of granulopenia, and in every instance the onset of the illness had been preceded by prolonged or intensive administration of drugs of this class, and they state, "In each of the 14 cases there was a definite history of amidopyrine in combination with a barbiturate or pyramidon in practically all of the cases we have seen."

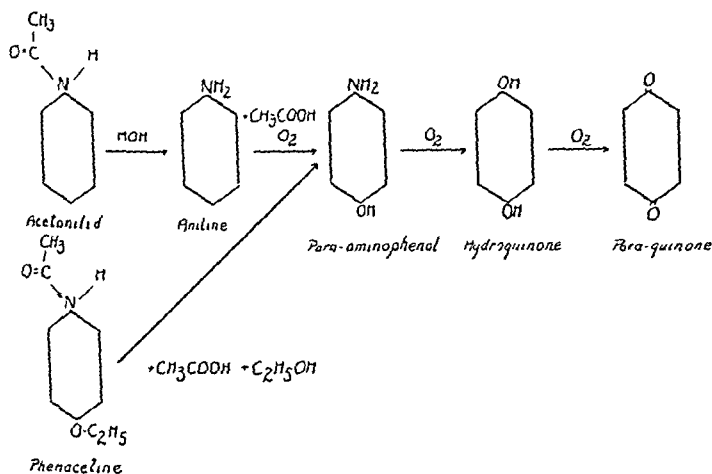
On the basis of the evidence submitted in this paper we, therefore, have formulated the hypothesis that the disease owes its high incidence in nurses and physicians to their indiscriminate use of drugs containing the benzamine group, the more prominent members of which are shown in Fig. 1 where the possible mechanism of their oxidation to the intermediate products is graphically illustrated.

It has been shown by repeated animal experiments that the subcutaneous injection of benzene will produce a depression of the bone marrow with a blood picture in which the leucocyte count falls to 200 or 300 cells with complete absence of granulocytes. It is our opinion that this effect is produced, not by the direct action of benzene upon the bone marrow, but by the action of some of its oxidation products. In support of this contention, rabbits were injected subcutaneously with a mixture of equal parts of benzene and olive oil and the tissues at the site of injection were analyzed eighteen hours later for some of these products. The findings showed benzene to be present only in traces, while phenol and catechol were present in considerable amounts. Analysis of a noninjected control animal showed these products to be absent. Similar results with the additional finding of small amounts of hydroquinone have been reported<sup>42</sup> by Schultzen and Naunyn, Munk, Baumann and Herter, and Nencki and Gaicosa. At the same time bone marrow from the injected animals was analyzed and showed the presence of both phenol and catechol but no trace of benzene. Similar control animals gave entirely negative results. This indicated that benzene as such did not reach the bone marrow but did not exclude further oxidation products of the benzene ring as the causa-



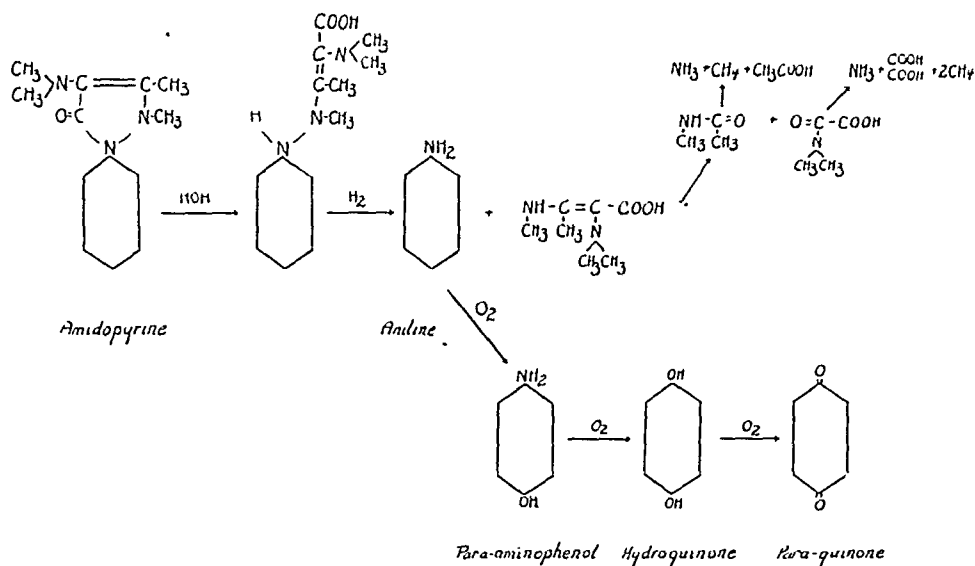
It is well known that the presence of an amine group on the benzene ring greatly increases the ease with which the structure can be oxidized. This property is little influenced by the substitution of additional side chains either upon the ring or upon the amine group itself. With these facts in view, we have worked out the possible oxidation products of these drugs in an attempt to arrive at an end-product common to both benzene and the benzamine compounds. These reactions are logical and sound chemically, but are highly theoretical physiologically, in that they have not been proved so to take place in the animal organism.

The oxidation of acetanilid and phenacetine to para-aminophenol both in vitro and in vivo<sup>44</sup> is well established. The compound has been isolated from the urine<sup>45</sup> and the blood serum<sup>46</sup> of patients to whom these drugs have been administered. The further oxidation of para-aminophenol to quinone<sup>47</sup> is easily carried out in vitro, but as yet, has not been demonstrated in animal metabolism. Hydroquinone is probably formed as an intermediate in this oxidation. These reactions are attended by the splitting off of acetic acid in the case of acetanilid, and of acetic acid and ethyl alcohol in the case of phenacetine. The side chains probably are oxidized further as straight chain carbon compounds. The complete reaction is as follows:



The literature affords little information as to the ultimate fate of amidopyrine in normal or abnormal animal metabolism. Current opinion is summed up in the statement that it is excreted either unchanged<sup>48</sup> or as related substances<sup>49</sup> but no mention is made as to what such related substances might be. Chemically, amidopyrine is a pyrazolon derivative of benzene, or it may be looked upon as a derivative of phenylhydrazine, since this compound is used in its synthesis. The presence of the ketone oxygen on the carbon adjacent to the nitrogen makes this linkage extremely unstable<sup>50</sup> so, it is at this point that the pyrazolon ring would break. This gives rise to a straight chain carbon substitution product of phenylhydrazine. The linkage between the two nitrogens of any phenylhydrazine derivative is easily broken by reduction,<sup>51</sup> regardless of the nature of the substituted side chain. This reaction would

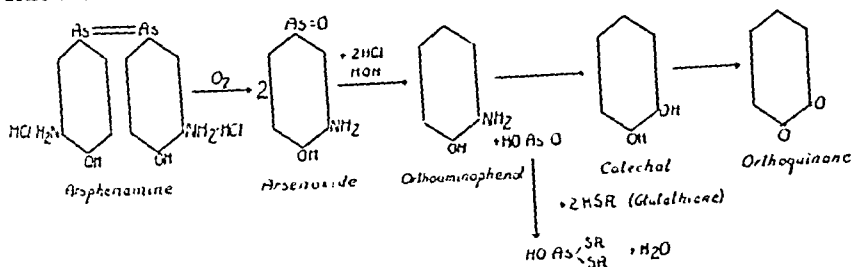
give rise to anilin plus a side chain which may be disposed of as indicated in the following reactions. The anilin thus formed is readily oxidized in vitro to para-aminophenol,<sup>44</sup> hydroquinone and finally to quinone, and therefore might undergo the same reaction in the human body. These reactions are as follows:



Several aspects of the toxicity of arsphenamine have been dealt with by Voegtlin.<sup>52</sup> He explains these either on the physical changes that the drug undergoes when injected into the blood stream, or upon the chemical action of trivalent arsenic with glutathione. It is pointed out that this latter reaction upsets the normal tissue oxidation-reduction equilibrium with a resulting tissue asphyxiation. He mentions the occurrence of "aplastic anemias" in some instances, subsequent to the administration of arsphenamine, but does not explain this marrow depression other than by saying that it may be due to the longer retention of arsenic in the bone marrow than in any other tissue.<sup>53</sup> However, our animal experiments in which trivalent arsenic in the form of Fowler's solution was administered over a period of forty days produced no depression.

This lack of action of arsenic tended to center attention upon the benzamine nucleus of the compound. It is well established that arsphenamine or neoarsphenamine when administered intravenously, is readily decomposed by breakage of the double bond between the arsenic atoms, with the resulting formation of two molecules of arsenoxide.<sup>52</sup> According to Voegtlin, it is this compound which, by virtue of its trivalent arsenic, reacts with the SH of glutathione. He also shows that arsenous acid ( $\text{HO}-\text{As}=\text{O}$ ) when administered to animals, is more toxic than either arsphenamine or arsenoxide.<sup>54</sup> This would point to the probability that trivalent arsenic may exert its toxic effect equally well regardless of whether or not it is attached to the benzene ring. Therefore, removal of the arsenic from the ring with its hydrolysis to arsenous acid would leave the benzamine group, ortho-aminophenol, free to

be oxidized to catechol, and finally to ortho-quinone. On the other hand, if arsenic is not split from the ring, the hydroxy and the amine groups can be oxidized, without interference, to the quinone form. This chain of reactions is as follows:



From the above reactions, it is obvious that the progressive oxidation of benzene, acetanilid, phenacetine, amidopyrine, and arspenamine gives rise to several compounds in common. These intermediate and end-products are hydroquinone, catechol, orthoquinone, and para-quinone. It is our belief that one or more of these compounds are responsible for the depressant action of benzene upon the bone marrow, and if so, would point to the benzamine group as also being primary agents in the production of granulopenia.

It must be noted in Table VII that a large percentage of persons in these groups used acetylsalicylic acid in the form of either aspirin or empirin. This compound has as its basis the benzene ring, but no attached amine group. It is well established<sup>55</sup> that this drug is partly hydrolyzed in the gastrointestinal tract to salicylic acid. The unhydrolyzed portion is largely excreted as such, while the salicylic acid is excreted mainly unchanged or conjugated with sulphuric and glycuronic acid. A small portion may be oxidized to oxysalicylic acid and hydroquinone. The amount of this latter product would probably be very small since from a chemical standpoint, the absence of the amine group on the ring would be responsible for the slight degree to which this reaction would take place in comparison to the degree of occurrence of this same reaction in the benzamine drugs.

From a study of the various chemical reactions involved, it becomes apparent that the therapeutic effect of most of these drugs is based on one of the oxidation products. It is also true that the bone marrow depressing effect of benzene is due to one of its oxidation products and not to the drug itself. Therefore, it seems probable that bone marrow depression should be produced by the intravenous injection of one or more of these products.

Up to this time we have been able to produce a mild grade of leucopenia in two laboratory animals with the use of hydroquinone as previously reported.<sup>23</sup> At this time we have injected a large series of animals intravenously with hydroquinone, catechol, aniline, para-aminophenol, quinone and phenol, and we have been able to produce mild and severe grades of neutropenia only in an occasional animal and not to such an extent that we are able to state with certainty that we have reproduced the clinical picture. It must be remembered that all laboratory animals do not have inferior bone marrows, and the same situation may exist in the rabbit as we know exists in the

human being, that is, only an occasional human being develops granulopenia, while many thousands of individuals have taken the benzamine drugs indiscriminately and have shown no depression of the bone marrow.

This can well be illustrated in the case of arsphenamine. As shown by the statistics of the Medical Department of the United States Navy<sup>56</sup> nearly 700,000 doses of neoarsphenamine were administered in the eight-year period from 1925 to 1932, inclusive, with untoward reactions totaling 519, or one reaction in 25,000 doses, with only one bone marrow depression to each 90,000 injections. This is equally true of granulopenia, since only 500 cases have been reported in the United States from a population of 120,000,000 people. Therefore, an inferior, weakened bone marrow must be presupposed in the production of this disease. By the use of various oxidation products, we have been able to depress the leucocyte count of rabbits to as low as 1,000 cells per cubic millimeter. Detailed results of these experiments will be presented in a separate communication.

#### CONCLUSIONS

1. The etiology of granulopenia has not yet been proved.
2. It seems reasonable that a certain percentage of the cases is due to arsphenamine, a certain percentage to gold salts and perhaps other chemicals, leaving, however, a large group of the idiopathic type with an unknown etiology.
3. The disease is more prevalent among physicians and their relatives, nurses, hospital employees and members of the allied professions, than in any other group of people in the United States.
4. It is essentially a disease of the white race.
5. It affects primarily the better class of our people.
6. Its distribution is correlated with the usage of benzamine drugs.
7. A record of 11 cases is presented in which the clinical onset was preceded by prolonged or intensive administration of drugs containing the benzamine group.
8. Statistics are presented to show that this class of drugs has wide usage among members of the medical and allied professions.
9. An hypothesis for its production, based on oxidation reactions of the benzamine drugs, is presented.
10. In the clinical or experimental development of granulopenia, it is necessary to presuppose the existence of a previously weakened, damaged, or idiosyncratic bone marrow which may be congenital or acquired.
11. It is urged that all who have the opportunity to study cases of granulopenia should direct their attention to a careful history of known marrow depressing agents and the usage of benzamine drugs in particular.

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## BLOOD IODINE STUDIES\*

### II. THE NORMAL IODINE CONTENT OF HUMAN BLOOD

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#### INTRODUCTION

THE constant occurrence of iodine in normal human blood is now firmly established. The ample demonstration of this fact has followed the development of modern micromethods, designed particularly to detect the minute amounts consistently present in clinically obtainable quantities of blood. It is apparent, particularly from the extensive work of Sturm<sup>1</sup> and that of Lunde and his associates,<sup>2</sup> as well as from our own studies,<sup>3</sup> that there is a definite interrelationship between thyroid activity and the blood iodine *niveau*. Furthermore, it is evident that this *niveau* is altered by other physio-

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logic conditions, notably menstruation and pregnancy. There is thus opened up a new approach to studies of thyroid function in health and in disease.

It is evident that it is essential to establish the normal range and average level of the blood iodine in normal individuals, as well as in patients without thyroid disease. Such studies are now available. On the other hand, extensive investigation of the iodine deficiency theory of goiter has led to the conclusion that the iodine intake varies in different regions. Likewise, the manifestations of thyroid disease vary widely in different countries. Too, available iodine, as existing in the food, water, and air, is not the same in various geologic regions. As a consequence it has seemed advisable to make blood iodine determinations of normal individuals in this region; and particularly of our hospital and out-patients without thyroid disease.\* We wish to present in this paper these findings, together with a review of the pioneer work on the blood iodine, and a comparison of our results with those of the more recent studies.

#### HISTORICAL

Baumann's<sup>4</sup> discovery, in 1895, of the presence of considerable iodine within the thyroid gland led to a renewed interest in iodine metabolism as related to thyroid function. Better methods for the determination of iodine were developed, and the blood and other tissues were analyzed for iodine.

The earliest data which we have found for the blood iodine are presented mainly for their historic interest, since they are for the most part at variance with those of the more recent studies. In 1899 Gallard<sup>2</sup> investigated the absorption of iodide by the skin, and its subsequent distribution in the blood and other tissues. He found the iodine content of rabbit blood, after bathing the shaved abdominal skin with sodium iodide solution, to be 635 gamma† per cent. This high value, however, is in accord with other later observations, since it has been shown that the blood iodine rises sharply after iodine administration.<sup>1</sup> In a normal control animal he found 420 gamma per cent. This figure is far too high, in the light of recent studies and of our own work, and indicates contamination, or that the animal had previously received iodine in some form. Gallard used 100 cc. of blood, and a modification of the Rouboudin-Baumann method devised by Bourcet.<sup>6</sup>

Gley and Bourcet,<sup>7</sup> unable to demonstrate iodine in 100 to 200 cc. samples, used a liter of dog's carotid blood to determine the blood iodine. Bourcet's<sup>6</sup> method was followed. They found from 0.013 mg. to 0.112 mg. per liter, or a range of from 1.3 to 11.2 gamma per cent, and an average of 5.5 gamma per cent for the seven dogs. No correlation was observed between the amount of iodine present in the thyroid gland and the blood iodine level. In clotted blood they found the iodine in the serum, and none in the clot. In centrifuged blood the iodine was found only in the plasma. This was not confirmed by Veil and Sturm.<sup>1</sup> Dialysis of the serum or plasma revealed that no iodine

\*The determinations reported at this time are all from the Billings Hospital of the University of Chicago.

†A gamma is the unit used to designate the minute amounts of iodine present in the blood and tissues. It represents one one-thousandth of a milligram (0.001 mg.). The unit has also been designated a microgram.

passed into the dialysate. They consequently concluded that the iodine existed in the plasma in protein combination, and that it was a normal constituent of the blood.

Bourcet<sup>8</sup> investigated the iodine content of other organs and tissues. He found 0.005 mg. of iodine in 200 c.c. of carotid blood pooled from three male rabbits. This is 2.5 gamma per cent. He also found small quantities of iodine in dog's blood. In studying iodine elimination, he examined human menstrual blood. In five individuals this contained from 0.80 to 0.90 mg. of iodine per kilo of blood, or 80 to 90 gamma per cent. In a sixth woman simultaneous epistaxis blood was examined. The menstrual blood contained 94 gamma per cent of iodine, the epistaxis blood contained but 2.1 gamma per cent. He consequently concluded that menstrual blood is an important vehicle of iodine elimination, owing to its high iodine content.

Gley and Bourcet<sup>9</sup> subsequently investigated the effect of exsanguination on the blood iodine of male dogs, using from one-half to one liter of arterial blood. In six normal animals the blood iodine ranged from 2.8 to 9.8 gamma per cent, averaging 7.5 gamma per cent. In two others extreme values were found, 300 gamma per cent in one and less than 1 gamma per cent in another. They found that the blood iodine diminished rapidly after extensive exsanguination, and that after several days, it disappeared completely from the blood. These results are open to serious question.

Blum<sup>10</sup> sought to determine whether the specific "Jodeiweiss" of the thyroid was secreted into the blood stream. He regarded the thyroid as essentially an organ of intraglandular detoxication. In accord with his view the iodine, which Baumann had demonstrated to be present, might be used only in the local economy. With Grützner,<sup>11</sup> a method was devised and tested for the determination, by titration, of organically combined iodine in the presence of organic matter. The "inorganic" iodine was first extracted by acetone. This method was first applied to sheep's blood.

Blum and Grützner<sup>12</sup> subsequently attempted to answer the question of the constant presence of thyroid secretion, as organically combined iodine, in the blood. Using Hunter's<sup>14</sup> method on 50 to 1340 c.c. samples of whole blood, they found from 14 to 140 gamma per cent in five male sheep. However, in four others they failed to detect iodine. They likewise found none in two 125 c.c. samples of goat's blood. Using their own method<sup>11</sup> they detected iodine ("inorganic") but twice in the acetone extract of 6 samples of sheep's blood, and found none at all in the residual coagulum. Likewise no iodine was detected in the blood of 3 dogs, in the blood of 2 men, or in the blood of 2 pregnant women. In the blood of 5 dogs, "jodfrei ernährte," no iodine was found, even though the thyroids contained considerable iodine. They consequently doubted the existence of iodine as a normal constituent of the blood. After giving from 0.5 to 2.0 gm. of sodium iodide to 6 dogs, the blood constantly contained iodine, even up to twenty days after the administration. This was found in the acetone filtrate ("inorganic").

Pathologic bloods were then examined. Unfortunately they were unable to obtain and analyze the blood of patients with exophthalmic goiter, whose blood iodine was subsequently discovered<sup>1</sup> to be high. Among 16 patients

with eclampsia, 10 showed from a trace to 100 gamma per cent of organically combined (coagulum) iodine. Iodine was similarly found in the blood of 2 patients with severe nephritis and in one with convulsions due to a brain tumor. They concluded that iodine in organic combination does not occur in normal blood, and that the "inorganic" iodine occasionally found, comes from the food ingested, or from medication.

Cameron<sup>13</sup> made an extensive investigation of the iodine content of various plant and animal tissues. He used Hunter's method.<sup>11</sup> He found no iodine in dog or rabbit blood, nor in many other tissues. The sample, 0.5 gm., taken for analysis, however, was too small, in view of the minute amount of iodine normally present and the sensitiveness of the method he employed. It would be difficult to demonstrate the presence of iodine in 0.5 gm. of blood even by the most sensitive modern methods.

These pioneer investigations had shown that iodine may be detected by using sufficient amounts of normal mammalian blood and by employing adequate methods. A number of significant questions had thus been raised, among them: Is iodine a constant constituent of mammalian blood? If so, in what combination does it exist—organic, inorganic, or both? What is its relation to the thyroid gland, and to the thyroid iodine? What is its relation to thyroid disease? The answer to these, as well as further progress, was dependent upon the development and application of more sensitive methods for the biochemical determination of iodine.

"The first modern figure for blood iodine," as Orr and Leitch<sup>15</sup> are pleased to term it, is that of Kendall and Richardson<sup>16</sup> for ox blood, 13 gamma per cent.\* Kendall's method<sup>17</sup> was used on 100 c.c. samples. In twenty-three analyses, the level ranged from 10 to 17 gamma per cent, averaging 13 gamma per cent. In sixteen subsequent analyses, known amounts of iodine were added to the water solution after charring and fusing the blood. The amount added was subsequently subtracted from that determined. The range in these was from 4 to 21 gamma per cent. The average, however, was the same, 13 gamma per cent. When known amounts of iodine were added to whole blood, from 2.2 to 3.0 per cent was not recovered by the analysis. The lower limit of detectable iodine by Kendall's method is about 5 gamma (0.005 mg.).

Gley and Cheymol, observing that the goat thyroid contained considerable iodine,<sup>19</sup> examined the efferent blood from the thyroid veins of eight female goats.<sup>18</sup> In this manner they attempted to demonstrate the passage into the blood of the iodine-containing thyroid secretion. Kendall's method was used on 100 c.c. samples. The carotid blood was found to contain from 4.3 to 26 gamma per cent, averaging 12.3 gamma per cent. Efferent thyroid blood, however, was found to contain from 5 to 50 gamma per cent, averaging 20.8 gamma per cent. They consequently concluded that they had demonstrated the passage of the internal secretion of the thyroid into the blood stream. No correlation was observed between the varying iodine content of the thyroid gland and that of the efferent blood.

\*This is variously quoted in the literature as for human or dog blood. Kendall<sup>16</sup> used ox blood for the determinations. A large series of determinations were also made on human and dog blood. The values were all within a narrow range.

Cheymol and Gley,<sup>20</sup> using Kendall's method, found 13 gamma per cent of iodine in the pooled blood from 5 male guinea pigs, and 11 gamma per cent in the pooled blood of 5 females. In dog's carotid blood they found 9.6 gamma per cent, and in the blood of the female goat 12.4 gamma per cent. Samples of 100 c.c. were used for analysis.

#### RECENT INVESTIGATIONS

During the first decade of the twentieth century advancing goiter research had created a demand for an adequate method for the determination of minute amounts of iodine. In 1922 the Swiss Goiter Commission consequently requested von Fellenberg, of the Government Bureau of Hygiene, to develop such a method. He devised, perfected, and eventually synthesized the known existing quantitative procedures into a true micromethod for the determination of iodine.<sup>22</sup> The lower limit of detectable iodine by this method is about one ten-thousandth of a milligram (0.0001 mg.). Two of his pupils, Sturm and Lunde, soon applied his method to the study of the blood iodine in thyroid disease.

The successful application of the principles developed in von Fellenberg's method has led to such extensive developments that from this point we must limit ourselves to the discussion of normal human blood iodine values. Since determinations of the blood iodine on hospital patients have been used by some investigators as normal, we are including them in this report. However, we are omitting all blood iodine reports on goitrous patients, on patients with dietary deficiency diseases, on patients with acute inflammations, and on those with leucemia, as well as blood iodine determinations made on women during early menstruation, pregnancy, and lactation.

Alexander Sturm learned von Fellenberg's method at Berne. Returning to Veil's Clinic in Munich, he investigated extensively the blood iodine in normal individuals, in patients with thyroid disease, and in those with various other diseases. His study<sup>1</sup> presented, in 1925, the first comprehensive data of normal human blood iodine values. In a series of 36 individuals, studied in the late summer and fall, the blood iodine averaged 12.8 gamma per cent. In 24 normal individuals investigated during late fall and winter the average was lower, 8.3 gamma per cent. The iodine occurred in similar concentration in the plasma and in the corpuscles. When the blood proteins were precipitated by alcohol, about 35 per cent of the iodine was recovered in the alcoholic filtrate. The remaining 65 per cent was found in the precipitate. From Jena, in 1928, Sturm<sup>23</sup> reported a series of blood iodine determinations made on 10 men. These revealed high values in May and July, of 15.0 and 14.1 gamma per cent, respectively. The lowest level, found in December, was 9.9 gamma per cent.

Maurer and Diez,<sup>24</sup> of Munich, investigated the blood iodine during menstruation and pregnancy. During this study they determined the blood iodine of 10 women during the intermenstrual period. These determinations were made during the winter. They ranged from 4.2 to 15.0, averaging 9.2 gamma per cent. Later, Jahn and Kesselkaul,<sup>25</sup> also of Munich, studied the variation of the blood iodine during the menstrual cycle. They reported that 17 normal

women had intermenstrual blood iodine values ranging from 7.2 to 12.0 gamma per cent, and averaging 9.5.

Jansen<sup>26</sup> reported an average of 12 to 14 gamma per cent in normal individuals at Bonn.

In the study which DeQuervain and Smith<sup>27</sup> made at Berne, on the blood iodine of cretins and of patients with nontoxic nodular goiter, they included a series of determinations made on five normal individuals during the winter. These determinations ranged from 11.1 to 14.6 gamma per cent, with an average of 13.1.

Lunde, Closs and Pedersen,<sup>2</sup> reported 11 to 16 gamma per cent as the normal blood iodine level occurring in Oslo. For fractionating the blood iodine, Lunde devised a method of alcohol extraction. This differs from Sturm's<sup>1</sup> method of alcohol precipitation. By Lunde's method only 1 to 4 gamma per cent was found in the alcohol insoluble fraction. Work from Lunde's laboratory indicates that the alcohol insoluble fraction is of particular significance in the pathologic physiology of toxic goiter.

At Aberdeen, Orr and Leitch<sup>15</sup> determined the blood iodine by the Leitch and Henderson method. They found in 16 normal individuals, examined in the spring, an average of 6 gamma per cent, while that of 28 individuals, examined in the fall, averaged 8.4.

In 1929 Eisler and Schittenhelm,<sup>28</sup> using the von Fellenberg method, reported from 10 to 13 gamma per cent as the normal blood iodine level for Kiel. The same investigators in 1932,<sup>29</sup> reporting seventy-one blood iodine determinations, gave from 7 to 9 as the range for winter and from 9 to 12 as the range for the summer.

Fowweather,<sup>30</sup> while studying the blood iodine in patients with cancer, estimated the blood iodine in twelve normal individuals. He found a range of from 3.6 to 5.1 gamma per cent, with an average of 4.3. This investigation was made at Leeds, using the Leitch and Henderson method.

Baldauf and Pincussen,<sup>31</sup> who studied the interrelationship between the bromine and iodine concentration of the blood, reported an average of 10.9 gamma per cent for a series of blood iodine determinations made on normal individuals in Berlin. Scheringer,<sup>32</sup> also in Berlin, in a series of 9 individuals studied between January and July, found from 6 to 15 gamma per cent, with an average of 8 gamma per cent. The following year he reported<sup>33</sup> another group of 8 individuals with a range of 10 to 13. Twelve of Scheringer's 17 subjects were women and the intermenstrual values were taken as the normal for them.

In 1930 Turner,<sup>34</sup> of Detroit, reported 9 cases on which he found a range of from 9 to 15, and an average of 11 gamma per cent. He used his own method for the determination of the blood iodine.

Schneider and Widmann,<sup>35</sup> of Freiburg, used von Fellenberg's method on 15 patients, 5 normal subjects and 10 nongoitrous patients. They found the blood iodine to range from 8.2 to 17.5 gamma per cent, with an average of 12.5. A year later Widmann<sup>35</sup> used his own modification of the Schwaibold

closed oxidation procedure for blood iodine on 6 nongoitrous patients. He found blood iodine values ranging from 27 to 42 gamma per cent, with an average of 32. This modification needs further investigation to rule out the possibility of other oxidizing substances than iodine containing radicles being carried over in the oxidation process, and later reacting with the added potassium iodide.

Billmann<sup>37</sup> found a range of 8 to 18 gamma per cent in 10 individuals. The patient on whom Bøe and Elmer<sup>38</sup> studied the effect of intravenous thyroxin administration had a normal blood iodine of 12 gamma per cent.

An extensive study of the blood iodine fractions in normal subjects was reported from Paris by Nitzescu and Binder.<sup>39</sup> Their investigations were made on a group of healthy male subjects ranging in age from twenty to thirty years. The lowest average blood iodine, 8.7 gamma per cent, was found in 47 cases studied in February. The highest averages were found in June, 12.7 for 16 cases, and in July, 12.9 for 11 cases. The average inorganic fraction ranged from 2.2 gamma per cent in January to 5.1 gamma per cent in June, while the organic fraction was lowest in February at 6.1 and highest in July at 8.1 gamma per cent. There was thus a greater variation in the inorganic than in the organic fraction. The separation was carried out according to Lunde's procedure. In this determination the "organic" fraction contains all of the alcohol insoluble fraction plus the "organic" portion of the alcohol soluble fraction.

While studying the relationship of the iodine concentration in the blood to that in the cerebrospinal fluid, Hahn and Schurmeyer<sup>40</sup> found in 17 individuals a range of 7 to 14 gamma per cent, with an average of 10.6.

Dodds, Lawson and Robertson,<sup>41</sup> working in London and using Lunde's method, found in 7 normal subjects under forty years of age, an average of 10.2 gamma per cent with a range of from 7.8 to 17.6. The alcohol soluble fraction in the 7 varied from 4.9 to 11.9 gamma per cent, with an average of 7.7. The alcohol insoluble fraction in 16 cases varied from 2.4 to 5.8 gamma per cent, with an average of 4.

#### OBSERVATIONS .

The blood iodine determinations here reported were all made by Davis<sup>42</sup> method. This is a development of the von Fellenberg procedure. The results obtained are best presented in tabular form (Tables I, II and III).

In Table I is presented a series of 8 determinations made on 6 normal members of the Billings Hospital staff. The determinations on the women were all made during the intermenstrual period. These individuals were all taking the usual hospital diet, which contains no iodized salt. The range of the 8 determinations is between 8.9 and 13.8, and the average 12 gamma per cent.

In Table II is presented a series of 13 determinations made on 10 hospital patients. The diagnoses are given. None of these patients revealed evidence of thyroid disease. All were under careful observation, and on controlled diets which contained no iodized salt. The age range is wide, from thirteen

to seventy-eight years. The blood iodine ranges from 8.5 to 14.4, averaging 12.3 gamma per cent. In accord with Fowweather<sup>30</sup> we observe that the presence of cancer has no obvious effect upon the level of the blood iodine.

TABLE I  
BLOOD IODINE IN NORMAL INDIVIDUALS ON USUAL HOSPITAL DIET

| CASE                  | AGE | SEX | BLOOD IODINE<br>GAMMA PER CENT | DATE     |
|-----------------------|-----|-----|--------------------------------|----------|
| Technician<br>Chemist | 24  | M   | 11.8                           | 9/ 9/30  |
|                       |     |     | 13.3                           | 10/ 6/31 |
|                       | 25  | F   | 13.8                           | 1/ 8/32  |
| Nurse<br>Nurse        |     |     | 8.9                            | 11/16/31 |
|                       | 25  | F   | 13.8                           | 11/ 5/31 |
|                       | 28  | F   | 9.3                            | 11/18/31 |
| Nurse<br>Student      |     |     | 12.3                           | 4/20/31  |
|                       | 29  | F   | 13.1                           | 1/10/32  |
|                       | 30  | F   |                                |          |

TABLE II  
BLOOD IODINE IN HOSPITAL PATIENTS WITHOUT THYROID DISEASE

| CASE                    | AGE | SEX | DIAGNOSIS             | BLOOD IODINE<br>GAMMA PER CENT | DATE    |
|-------------------------|-----|-----|-----------------------|--------------------------------|---------|
| 36112<br>22859          | 13  | M   | Obesity               | 10.4                           | 4/ 6/31 |
|                         |     |     |                       | 13.9                           | 7/ 2/30 |
| 24761<br>17938          | 20  | M   | T. B. spine           | 13.5                           | 9/ 5/30 |
|                         |     |     |                       | 8.5                            | 7/ 7/30 |
|                         | 27  | F   | Paronychia            | 12.0                           | 8/ 4/30 |
| 32540<br>22940          | 33  | F   | Chronic osteomyelitis | 14.1                           | 8/18/30 |
|                         |     |     |                       | 12.5                           | 1/ 8/31 |
|                         | 41  | M   | Anal ulcer            | 13.5                           | 7/ 2/30 |
| 40972<br>41561<br>41128 | 52  | M   | Fracture tibia        | 11.8                           | 8/ 5/30 |
|                         |     |     |                       | 11.2                           | 7/ 3/31 |
|                         | 57  | F   | Melanosarcoma         | 14.4                           | 7/ 7/31 |
| 25863                   | 57  | M   | Carotid body Ca.      | 14.4                           | 7/ 9/31 |
|                         | 68  | M   | Ca. of mouth          | 9.9                            | 8/ 1/30 |
|                         | 78  | M   | Ca. of pharynx        |                                |         |

TABLE III  
BLOOD IODINE IN OUT-PATIENTS WITHOUT THYROID DISEASE

| CASE  | AGE | SEX | DIAGNOSIS                    | BLOOD IODINE<br>GAMMA PER CENT | DATE    |
|-------|-----|-----|------------------------------|--------------------------------|---------|
| 27006 | 6   | M   | Normal                       | 12.7                           | 8/14/30 |
| 28169 | 16  | F   | Infantile paralysis          | 16.2                           | 9/15/30 |
| 30393 | 23  | F   | Retroverted uterus           | 12.7                           | 3/12/31 |
| 24761 | 27  | F   | Paronychia                   | 11.2                           | 8/ 7/30 |
| 28425 | 29  | F   | T. B. cervical lymphadenitis | 8.5                            | 9/19/30 |
| 28499 | 30  | F   | T. B. cervical lymphadenitis | 9.2                            | 9/22/30 |
| 28500 | 31  | M   | Furuncle of arm              | 10.6                           | 9/22/30 |
| 24531 | 31  | F   | "Neurasthenia"               | 9.3                            | 7/14/30 |
| 27651 | 41  | M   | Chronic spondylitis          | 9.5                            | 9/ 4/30 |
| 30545 | 43  | M   | Chronic osteomyelitis        | 12.7                           | 3/16/31 |
|       |     |     |                              | 11.8                           | 3/23/31 |
| 31527 | 46  | F   | Cervical lymphoblastoma      | 12.7                           | 3/23/31 |
| 27653 | 48  | M   | Tibial tuberculid            | 11.4                           | 9/ 4/30 |

In Table III is presented a series of 13 determinations made upon 12 ambulatory patients visiting the out-patient clinic. All presented no evidence of thyroid disease. None was using iodized salt. The range is from 8.5 to 16.2 and the average is 11.4 gamma per cent. The range is thus somewhat

TABLE IV  
NORMAL HUMAN BLOOD IODINE

| OBSERVER                                | DATE | LOCALITY | METHOD   | RANGE     | AVERAGE | NUMBER OF CASES | SEASON               |
|-----------------------------------------|------|----------|----------|-----------|---------|-----------------|----------------------|
| Veil and Sturm <sup>1</sup>             | 1925 | Munich   | v. F.*   |           | 12.8    | 36              | Late Summer and Fall |
|                                         | 1927 | Munich   | v. F.    | 4.2-15    | 8.3     | 24              | Late Fall and Winter |
| Maurer and Diez <sup>24</sup>           |      |          |          |           | 9.2     | 10              | Winter               |
|                                         |      |          |          |           | 10.4    | 10              | November             |
| Sturm <sup>23</sup>                     | 1928 | Jena     | v. F.    |           | 9.9     | 10              | December             |
|                                         |      |          |          |           | 11.0    | 10              | January              |
|                                         |      |          |          |           | 15.0    | 10              | May                  |
|                                         |      |          |          |           | 14.1    | 10              | July                 |
|                                         |      |          |          |           | 12.1    | 10              | September            |
|                                         |      |          |          |           | 10.4    | 10              | November             |
| Jansen <sup>25</sup>                    | 1928 | Munich   | v. F.    | 12-14     |         |                 |                      |
| DeQuervain and Smither                  | 1928 | Berne    | v. F.    | 11.1-14.6 | 13.1    | 5               | Winter               |
| Jahn and Kesselkaul <sup>25</sup>       | 1928 | Munich   | v. F.    | 7.2-12    | 9.5     | 17              |                      |
| Lunde, Gloss and Pedersen <sup>26</sup> | 1929 | Oslo     | v. F.    | 11-16     |         |                 |                      |
| Orr and Leitch <sup>15</sup>            | 1929 | Aberdeen | L. & IL. |           | 6.0     | 16              | Spring               |
| Eisler and Schittenhelm <sup>28</sup>   | 1929 | Kiel     | v. F.    | 10-13     | 8.4     | 28              | Fall                 |
| Fowweather <sup>30</sup>                | 1930 | Leeds    | L. & IL. | 3.6-5.1   | 4.3     | 12              |                      |



TABLE IV—CONT'D

| OBSERVER                                  | DATE | LOCALITY | METHOD | RANGE    | AVERAGE | NUMBER OF CASES | SEASON    |
|-------------------------------------------|------|----------|--------|----------|---------|-----------------|-----------|
| Baldauf and Pincussen <sup>41</sup>       | 1930 | Berlin   | v. F.  |          | 10.9    |                 |           |
| Scheringer <sup>42</sup>                  | 1930 | Berlin   | v. F.  | 6-15     | 8.0     | 17              | Winter    |
| Turner <sup>44</sup>                      | 1930 | Detroit  | Own    | 9-15     | 11.0    | 9               |           |
| Scheringer <sup>43</sup>                  | 1931 | Berlin   | v. F.  | 10-13    |         | 8               | Winter    |
| Schneider and Widmann <sup>35</sup>       | 1931 | Freiburg | v. F.  | 8.2-17   | 12.5    | 15              | Fall      |
| Bilman <sup>37</sup>                      | 1931 |          |        |          |         |                 |           |
| Bae and Elmer <sup>38</sup>               | 1931 | Berlin   |        | 8-18     | 12.0    | 1               |           |
| Nitzescu and Binder <sup>39</sup>         | 1931 | Paris    | v. F.  |          | 8.4     | 19              | January   |
|                                           |      |          |        |          | 8.7     | 47              | February  |
|                                           |      |          |        |          | 9.4     | 41              | March     |
|                                           |      |          |        |          | 12.7    | 16              | June      |
|                                           |      |          |        |          | 12.9    | 11              | July      |
| Schittenhelm and Elster <sup>29</sup>     | 1932 | Kiel     | v. F.  | 7-9      | 11.4    | 29              | September |
|                                           |      |          |        |          | 11.3    | 10              | December  |
|                                           |      |          |        |          |         | 71              | Winter    |
|                                           |      |          |        |          |         |                 | Summer    |
|                                           |      |          |        |          | 32.0    | 6               |           |
| Widmann <sup>36</sup>                     | 1932 | Freiburg | Own    | 27-42    |         |                 |           |
| Hahn and Schurmeyer <sup>40</sup>         | 1932 |          |        |          |         |                 |           |
| Dodds, Lawson and Robertson <sup>41</sup> | 1932 |          |        |          |         |                 |           |
| Davis, Curtis, and Cole                   | 1932 | London   | v. F.  | 7-14     | 10.6    | 17              |           |
|                                           | 1932 |          | v. F.  | 7.8-17.6 | 10.2    | 7               |           |
|                                           | 1934 | Chicago  | v. F.  | 8.5-16.2 | 11.9    | 28              |           |

\*v. F. = von Fellenberg.

L. &amp; H. = Lettich and Henderson.

greater. This may be due to the fact that there was no dietary control and that certain of the bloods were not drawn in the postabsorptive state.

In all, 34 determinations made on 28 individuals without evidence of thyroid disease revealed a range of from 8.5 to 16.2 and an average of 11.9 gamma per cent. It would thus appear that about 12 gamma per cent is the normal average blood iodine level in Chicago.

The 18 determinations made upon women ranged from 8.5 to 16.2, averaging 11.6 gamma per cent. The 16 determinations made upon men ranged from 9.5 to 14.4, averaging 12.2 gamma per cent. The greater range of the female levels is presumably to be associated with the cyclic variations in the blood iodine due to menstruation. The male average level is slightly higher.

#### DISCUSSION

The results of available studies upon the normal blood iodine are presented in Table IV. Omitting the very low results of Fowweather<sup>30</sup> and the very high results of Widmann,<sup>31</sup> the average normal levels for the blood iodine vary from 6.0 to 13.1 gamma per cent. Since iodine loss is the most important single factor in making the determinations by the open oxidation methods, we are inclined to regard those results at the upper range as more nearly the true condition. A study of the table would lead us to conclude that the blood iodine in normal individuals, or in patients with normal thyroid function, is only influenced slightly, if at all, by geographic location.

The seasonal variation of the blood iodine is of interest. It corresponds to that observed in the thyroid gland by Seidell and Fenger.<sup>13</sup> A tabulation of available data readily shows a confirmation of the results first announced by Veil and Sturm,<sup>1</sup> that the highest values are obtained in the summer months and the lowest in the winter.

The fractionation of the blood iodine into an alcohol or acetone soluble, and an insoluble fraction is doubtless of significance. We have not yet attempted this procedure. It is apparent that the alcohol insoluble fraction is approximately the same when the determination is consistently made by the same method. It varies greatly, however, with the mode of extraction. The work of Lunde and his associates on the blood iodine in hyperthyroidism points to the significance of the alcohol insoluble fraction. Presumably it is the thyroid hormone. The "inorganic" iodine may come from the diet or from catabolism of the "organic" iodine compounds.

#### SUMMARY

Iodine is constantly present in the human blood. The average level is about 12 gamma per cent. Development of our knowledge regarding this fact followed the institution of adequate micromethods for the determination of the minute amounts normally present. The blood iodine varies seasonally, being higher during the summer and lower during the winter months. The blood iodine may be separated into "organic" and "inorganic" fractions. Determinations of the blood iodine open up a new approach to the study of thyroid function in health and in disease.

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## THE SEDIMENTATION RATE AND POLYMORPHONUCLEAR COUNT IN RHEUMATOID AND MIXED ARTHRITIS\*

THEIR VALUE AS AN INDEX TO ACTIVITY

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THE sedimentation rate and the polymorphonuclear count (hereafter referred to as the nuclear count) have been applied in the study of a number of different diseases, but it has been only during the past few years that they have been used in the study of arthritis. A number of papers have been published on the value of the sedimentation rate in arthritis but comparatively few have appeared on the nuclear count.

Various investigators<sup>1, 2, 3, 4</sup> have stated that the sedimentation rate is greatly increased in rheumatoid arthritis while in osteoarthritis the rate is usually normal or only slightly increased. It has been used mostly as an aid in differentiating these two types of arthritis. It has been reported<sup>3</sup> that there is a close relationship between the sedimentation rate and the severity of the infection; that remissions are associated with a decreased rate and exacerbations with an increased rate. Some<sup>5, 6</sup> have suggested that this test may be of assistance in following the progress of the patient's illness. They have not, however, reported the frequency with which these changes occur. Kahlmeter<sup>1</sup> suggested the use of the sedimentation rate as an index of activity, a knowledge of which he believes to be essential for the proper use of physiotherapy. Forestier<sup>4</sup> has shown that as the patient improves, the sedimentation rate returns to normal.

The value of the nuclear count is based on the observation that an active infection is accompanied by an increase in the number of young polymor-

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phonuclear neutrophils in the blood. An increase in the number of these cells is termed "shift to the left." Only recently has this principle been used in the study of arthritis. Schilling<sup>7</sup> stated that in rheumatoid arthritis only slight changes are present in the hemogram, but other investigators using the Schilling hemogram have reported more conclusive findings. Cecil<sup>8</sup> reported a shift to the left in 52 per cent of 23 cases of rheumatoid arthritis and that Resnikoff found a shift to the left in 67 per cent of 28 cases. Eaton<sup>9</sup> in his study of 250 cases of chronic nontuberculous arthritis found a shift to the left in 90.4 per cent. Gerard and Boerner<sup>10</sup> reported two cases, and Piney<sup>11</sup> one, of arthritis with a shift to the left. Recently, Steinbrocker and Hartung,<sup>12</sup> using the filament nonfilament count, a modification of the Schilling hemogram advocated by Farley, St. Clair, and Reisinger, reported finding what is the equivalent of a shift to the left in 100 per cent of 50 cases of rheumatoid arthritis.

The present study was undertaken to determine whether exacerbations and the periods of improvement in patients with rheumatoid and mixed arthritis are accompanied by definite changes in the sedimentation rate and the nuclear count and the relationship between the two tests. We chose for our study a group of patients who attended the clinic regularly. At each visit a detailed record of both the subjective complaints and the objective findings was made. The presence of any intercurrent infections was also noted. Whenever there was a change in the condition of the patient, the sedimentation rate and nuclear count were determined. In a number of instances these determinations were made when there was no change in the patient's condition. They were always made at approximately the same time of the day. Westergren's<sup>13</sup> modification of Fahraeus' technic was used for the sedimentation, and a rate of 12 mm. or less per hour was considered normal. In making the nuclear counts, the technic of Cooke and Ponder<sup>14</sup> was used. Farley, St. Clair, and Reisinger,<sup>15</sup> in reviewing the work of Arneth, Schilling, and Cooke and Ponder, state that the classification of Arneth is too complicated for practical use, and that the method of Schilling lacks the clear-cut differentiation which can be obtained by following Cooke's criterion. This criterion is as follows: "If there is any band of nuclear material except a fine filament of nuclear material connecting the different parts of a nucleus, that nucleus for the purpose of the count cannot be said to be divided." With this as a basis, the polymorphonuclear neutrophils were divided into five groups according to the number of segments. In determining the presence or absence of a shift to the left, Cooke and Ponder considered as an average normal count, 12 Type I, 25 Type II, 44 Type III, 15 Type IV, and 4 Type V cells per 100 polymorphonuclear neutrophils.

In the early part of this work we failed to realize the importance of intercurrent infections, but as the work proceeded we excluded all those tests which were made when the patient was suffering from such infections. Determinations were made in 50 cases each of rheumatoid and mixed arthritis. A total of 262 sedimentation tests were done in the former and 234 in the latter group. Of the patients with rheumatoid arthritis 89 per cent had a

sedimentation rate above normal. The average rate was 36 mm. per hour. In 53 per cent of the tests the values were above 30 mm. per hour. Of the 234 determinations in mixed arthritis, 64 per cent were above normal. The average rate in this group was 18 mm. per hour with only 15 per cent of the cases showing values above 30.

Of 258 nuclear counts made on 50 patients with rheumatoid arthritis, 68 per cent showed a shift to the left. In 23 per cent the counts were within normal limits and in 9 per cent there was a slight shift to the right. In the cases of mixed arthritis, 244 counts were made, of which 57 per cent showed a shift to the left, 36 per cent presented normal counts and 7 per cent showed a shift to the right.

In 210 instances in the rheumatoid group the sedimentation rate and the nuclear count were compared. In 77 per cent a shift to the left was associated with a high sedimentation rate. In 10 per cent there was a normal sedimentation rate and either a shift to the right or a normal count. In 6.5 per cent there was a normal count or a shift to the right accompanied by a high sedimentation rate, and in 6.5 per cent there was a shift to the left with a normal sedimentation rate. In the series of mixed arthritis, 190 comparisons were made. Of these, 58 per cent presented both a shift to the left and a high sedimentation rate, while 14 per cent showed a normal sedimentation rate and either a normal count or a shift to the right. In 23 per cent of the cases in this group, a shift to the left was accompanied by a normal sedimentation rate, and in 6 per cent there was a high sedimentation rate contrasted with either a normal count or a shift to the right (Table I).

TABLE I  
CORRELATION OF THE SEDIMENTATION RATE AND THE NUCLEAR COUNT

|                                                   | RHEUMATOID<br>PER CENT | MIXED<br>PER CENT |
|---------------------------------------------------|------------------------|-------------------|
| High sedimentation rate<br>Shift to the left      | 77.0                   | 58                |
| Normal sedimentation rate<br>Normal nuclear count | 10.0                   | 14                |
| High sedimentation rate<br>Normal nuclear count   | 6.5                    | 6                 |
| Normal sedimentation rate<br>Shift to the left    | 6.5                    | 22                |

In 274 instances the sedimentation rate, the nuclear count and the clinical condition of the patients were simultaneously recorded in order to study the value of these tests as an index to activity. In this group no differentiation was made between the rheumatoid and mixed cases. Of the total number, 119 were made when there was improvement in the clinical symptoms, 56 when they were worse, and 99 when they were unchanged. Of the 119 made during clinical improvement, 74 per cent showed a definite reduction in both the sedimentation rate and the degree of the shift to the left; 6 per cent showed a slight increase in the sedimentation rate and a further shift to the left; 9 per cent showed no change in either the sedimentation rate or the nuclear

count; 6 per cent showed no change in the nuclear count but a reduction in the sedimentation rate; 3 per cent showed an approach to normal in the nuclear count but no change in the sedimentation rate; 2 per cent showed an approach to normal in the nuclear count and a slight increase in the sedimentation rate. Of the determinations made when there was an exacerbation of the clinical symptoms, 65 per cent showed an increase both in the sedimentation rate and in the degree of the shift to the left, while 14 per cent showed a slight reduction in both. In 14 per cent there was an increase in the sedimentation rate but a decreased shift to the left; in 7 per cent there was no change in the nuclear count but a slight reduction in the sedimentation rate. Where the clinical condition was unchanged, 49 per cent showed no change in either test. In 24 per cent there was a slight reduction in the sedimentation rate and in the shift to the left; 4 per cent showed a slight reduction in the sedimentation rate and an increased shift to the left; 6 per cent showed a slight increase in the sedimentation rate and in the shift to the left; 3 per cent presented a slight increase in the sedimentation rate but no change in the nuclear count; 3 per cent showed no change in the sedimentation rate but a decreased shift to the left; 3 per cent gave an increased sedimentation rate and a decreased shift to the left; 3 per cent gave no change in the sedimentation rate but an increased shift to the left, and 5 per cent gave a decreased sedimentation rate and no change in the nuclear count. (Table II.)

TABLE II  
CHANGES IN THE SEDIMENTATION RATE AND THE NUCLEAR COUNT CORRELATED WITH  
CHANGES IN THE CLINICAL CONDITION

| SEDIMENTATION RATES AND NUCLEAR COUNTS | CLINICAL CONDITION   |                   |                       |
|----------------------------------------|----------------------|-------------------|-----------------------|
|                                        | IMPROVED<br>PER CENT | WORSE<br>PER CENT | UNCHANGED<br>PER CENT |
| Sedimentation rate decreased           |                      |                   |                       |
| 1. Shift to the left decreased         | 74                   | 14                | 24                    |
| Sedimentation rate increased           |                      |                   |                       |
| 2. Shift to the left increased         | 6                    | 65                | 6                     |
| Sedimentation rate unchanged           |                      |                   |                       |
| 3. Nuclear count unchanged             | 9                    |                   | 49                    |
| Sedimentation rate decreased           |                      |                   |                       |
| 4. Nuclear count unchanged             | 6                    | 7                 | 5                     |
| Sedimentation rate unchanged           |                      |                   |                       |
| 5. Shift to the left decreased         | 3                    |                   | 3                     |
| Sedimentation rate increased           |                      |                   |                       |
| 6. Shift to the left decreased         | 2                    | 14                | 3                     |
| Sedimentation rate decreased           |                      |                   |                       |
| 7. Shift to the left increased         |                      |                   | 4                     |
| Sedimentation rate increased           |                      |                   |                       |
| 8. Nuclear count unchanged             |                      |                   | 3                     |
| Sedimentation rate unchanged           |                      |                   |                       |
| 9. Shift to the left increased         |                      |                   | 3                     |
| Sedimentation rate alone increased     |                      |                   |                       |
| 10. Sedimentation rate alone increased | 8                    | 79                | 3                     |
| 11. Nuclear count alone increased      | 6                    | 65                | 12                    |
| 12. Sedimentation rate alone unchanged | 12                   |                   | 13                    |
| 13. Nuclear count unchanged            | 15                   | 7                 | 55                    |
| 14. Sedimentation rate alone decreased | 80                   | 21                | 57                    |
| 15. Nuclear count alone decreased      | 79                   | 28                | 33                    |
|                                        |                      |                   | 30                    |

## DISCUSSION

Although several articles have been published on both the sedimentation rate and the nuclear count in arthritis, there have been no reports of a correlation of the two tests and their value as indicators of activity. They have been used mainly as an aid in the differential diagnosis between rheumatoid and osteoarthritis. Our study indicates that these tests are of considerable value as an index of the activity of the arthritic process in patients with rheumatoid and mixed arthritis and as such are efficient aids in judging the progress of the patient. When the clinical condition is improved, there is usually a decrease in the sedimentation rate and a lessened shift to the left. Conversely, an increase in the severity of the arthritic process is associated with a rise in the sedimentation rate and a further shift to the left. The degree of change in these tests is fairly proportionate to the degree of change in the clinical condition. On numerous occasions we noted that intercurrent infections, such as colds and sore throats, adversely affected the sedimentation rate and nuclear count, and they, therefore, must be excluded before attributing changes in the tests to changes in the course of the disease. In the absence of any infection, however, the sedimentation rate and nuclear count tend to return to normal when the clinical symptoms have subsided. We have also observed that a hidden focus of infection will frequently give a persistent, high sedimentation rate and a shift to the left.

## CONCLUSIONS

1. In the majority of cases of rheumatoid and mixed arthritis the sedimentation rate and the nuclear count serve as an index of activity, and they may, therefore, be used as a means of determining the progress of these diseases.
2. The degree of change in the clinical symptoms is reflected in parallel changes in the sedimentation rate and nuclear count.
3. Intereurrent and focal infections adversely affect the sedimentation rate and nuclear count, and their presence must be excluded before changes in these tests may be attributed to variations in the activity of the arthritic process.
4. The variations in the sedimentation rate and the nuclear count do not correspond 100 per cent to the variations in the arthritic state, and therefore, neither alone is finally diagnostic; and knowledge of both the sedimentation rate and the nuclear count together should increase the accuracy of the interpretations.

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## THE BACTERICIDAL AND FUNGICIDAL ACTION OF HOMOLOGOUS HALOGEN PHENOL DERIVATIVES AND ITS "QUASI- SPECIFIC" CHARACTER\*

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### I. DERIVATIVES OF PARACHLOROPHENOL

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### INTRODUCTION

IN TWO recent papers<sup>1</sup> we described the preparation of a number of halogen phenol derivatives with bactericidal properties, giving also a survey of published work which preceded our investigations in this field. While all the derivatives studied by us were found to be germicidal toward a number of microorganisms among which were several resistant pathogenic bacteria and fungi, some of them were shown to possess this capacity to a remarkably high degree. In following up the relationship between the chemical structure and the bactericidal action of these compounds we observed a number of regularities, viz.:

1. Halogen substitution intensifies the microbicidal potency of phenol derivatives, the presence of halogen in the para-position to the hydroxyl group being more effective in this respect than in the ortho-position.

2. Introduction of aliphatic or aromatic groups into the nucleus of halogen phenols increases the bactericidal potency (up to certain limits), this increase depending, in the case of alkyl substitution, upon the number of carbon atoms present in the substituting group or groups.

3. As a rule the intensifying effect upon the bactericidal potency of a normal aliphatic chain with a given number of carbon atoms is greater than

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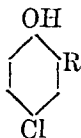
\*From the Plaut Research Laboratory, Lehn & Fink, Inc.  
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that of a branched chain, or of two or more alkyl groups with the same total number of carbon atoms.

4. Orthoalkyl derivatives of parachlorophenol are more actively germicidal than paraalkyl derivatives of orthochlorophenol.

5. In the case of the higher homologues the germicidal action manifests a "quasi-specific" character in that beginning with a definite point (which is different in the case of the various test organisms), with the further increase of the weight of the substituting groups, the germicidal capacity drops to almost total inactivity with respect to certain microorganisms (*Eberthella typhi*, *Eberthella paradysenteriae*), while rising to comparatively enormous values with respect to others (*Staphylococcus aureus*, streptococcus, *Mycobacterium smegmatis*).

It is the object of further work to extend the scope of our inquiry into the bacteriologic and pharmacologic phases of this problem, with the ultimate view of ascertaining the possible utility of these compounds for therapeutic purposes. The present paper furnishes the data obtained thus far with compounds derived from parachlorophenol; these compounds may be represented by the general formula



where R is an aliphatic or aromatic substituent.

The following microorganisms were used in this investigation: *Eberthella typhi*, *Escherichia coli*, *Eberthella paradysenteriae* (Flexner), *Salmonella schottmülleri* (*B. paratyphosus B*), *Staphylococcus pyogenes aureus*, streptococcus (hemolytic strain), *Micrococcus catarrhalis*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis* (hom.), *Mycobacterium tuberculosis* (avium), *Mycobacterium leprae* (mur.), *Mycobacterium leprae* (hom.), *Trichophyton roseaceum*, *Monilia albicans*, and *Achorion schönleinii*.

#### MONOALKYL DERIVATIVES

Tables I to IV illustrate the microbicidal action of the series of monoalkyl derivatives of parachlorophenol in relation to their chemical constitution, and more particularly to the increase in length (or weight) of the substituting side chain. Wherever possible, the minimum germicidal concentrations have been determined and the "phenol coefficients" calculated. Table I gives the results obtained with four gram-negative organisms of the typhoid colon group, in Table II the test organisms are (gram-positive and gram-negative) cocci, in Table III they are bacteria of the acid-fast group, while Table IV refers to three pathogenic fungi.\*

When considered individually, certain among the several compounds reveal a bactericidal effect of considerable intensity. However, in addition there is also unmistakable evidence of a parallelism in both a qualitative and

\*The figures given in parentheses are of relative significance only, as alcohol in excess of 10 per cent but below 20 per cent had to be used in order to produce the desired concentrations (owing to the very limited solubility of the respective compounds).

TABLE I

THE BACTERICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL UPON ORGANISMS OF THE TYPHOID-COLON GROUP.  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

|                  | EBERTHELLA TYPHI |        | EBERTHELLA PARADYS-<br>ENTERIAE |        | SALMONELLA SCHOTTHÜLLERI<br>(= PARATYPHOSUS B.) |        | ESCHERICHIA COLI |        |
|------------------|------------------|--------|---------------------------------|--------|-------------------------------------------------|--------|------------------|--------|
|                  | I                | II     | I                               | II     | I                                               | II     | I                | II     |
| p-Chlorophenol   | 1: 650           | 4.3    | 1: 700                          | 4.7    | 1: 600                                          | 4.3    | 1: 600           | 4.6    |
| Alkyl Radical    |                  |        |                                 |        |                                                 |        |                  |        |
| Methyl           | 1: 2,000         | 12.5   | 1: 2,000                        | 14.3   | 1: 1,800                                        | 12.9   | 1: 2,000         | 14.3   |
| Ethyl            | 1: 4,000         | 28.6   | 1: 4,500                        | 32.1   | 1: 4,000                                        | 28.6   | 1: 3,500         | 26.9   |
| n-Propyl         | 1:14,000         | 93.3   | 1:14,000                        | 100.0  | 1:10,000                                        | 714.0  | 1:12,000         | 85.7   |
| n-Butyl          | 1:22,500         | 141.0  | 1:25,000                        | 167.0  | 1:16,000                                        | 114.0  | 1:16,000         | 114.0  |
| n-Amyl           | 1:25,000         | 156.0  | 1:30,000                        | 200.0  | 1:14,000                                        | 100.0  | 1:20,000         | 154.0  |
| Sec. Amyl        | 1: 7,000         | 46.7   | 1:12,000                        | 80.0   | 1: 6,000                                        | 42.9   | 1: 5,000         | 41.7   |
| n-Hexyl          | (1: 3,250)       | (23.2) | 1:50,000                        | 333.0  | (1: 3,000)                                      | (21.4) | (1: 5,000)       | (35.7) |
| Cyclohexyl       | >1: 4,000        | <26.7  | 1:12,000                        | 75.0   | >1: 2,000                                       | <14.3  | >1: 2,000        | <14.3  |
| n-Heptyl         | (1: 3,000)       | (20.0) | (1: 3,000)                      | (20.0) | (1: 2,000)                                      | (14.3) | (1: 2,000)       | (14.3) |
| Phenol (control) | 1:140-150        | 1.0    | 1:140-160                       | 1.0    | 1:140-150                                       | 1.0    | 1:140-150        | 1.0    |

quantitative respect in the reaction toward the homologues of this series, of the microorganisms in Table I on one hand, and of those in the remaining three tables on the other.

Thus, in Table I the bactericidal efficacy rises with the increasing weight of the substituting side chain reaching a maximum with the n-butyl derivative in the case of *Salmonella schottmülleri*, and with the n-amyl derivative in the cases of *Eberthella typhi* and *Escherichia coli*, while the n-hexyl derivative is the most effective against *Eberthella paradysenteriae*. After reaching the maximum, one observes a rather abrupt drop of the bactericidal potency with the further increase of the weight of the substituents.

In agreement with observations made with other classes of phenol derivatives, here, too,<sup>2, 3, 4</sup> substitution by branched or cycloalkyl groups leads to compounds of lower bactericidal potency than that of the normal alkyl derivatives with the same number of carbon atoms in the substituting group.

An entirely different picture is furnished by Table II. Here, too, the coccidal potency increases with the increasing weight of the substituting radical, but its range extends beyond the turning points of the preceding table.

TABLE II

THE GERMICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL UPON PATHOGENIC COCCI.

MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

|                  | STAPHYLOCOCCUS<br>AUREUS |         | STREPTOCOCCUS<br>(HEMOL. STRAIN) |         | MICROCOCCUS<br>CATARRHALIS |        |
|------------------|--------------------------|---------|----------------------------------|---------|----------------------------|--------|
|                  | I                        | II      | I                                | II      | I                          | II     |
| p-Chlorophenol   | 1: 300                   | 4.3     | 1: 400                           | 4.4     | 1: 600                     | 4.0    |
| Alkyl Radical    |                          |         |                                  |         |                            |        |
| Methyl           | 1: 1,000                 | 12.5    | 1: 1,000                         | 11.1    | 1: 1,600                   | 10.7   |
| Ethyl            | 1: 2,750                 | 34.4    | 1: 2,500                         | 31.3    | 1: 4,000                   | 26.7   |
| n-Propyl         | 1: 7,500                 | 93.8    | 1: 7,000                         | 77.8    | 1: 12,000                  | 80.0   |
| n-Butyl          | 1: 18,000                | 257.0   | 1: 20,000                        | 250.0   | 1: 25,000                  | 167.0  |
| n-Amyl           | 1: 40,000                | 500.0   | 1: 50,000                        | 556.0   | 1: 40,000                  | 367.0  |
| Sec. Amyl        | 1: 25,000                | 312.0   | 1: 25,000                        | 312.0   | 1: 20,000                  | 142.0  |
| n-Hexyl          | 1: 100,000               | 1,250.0 | 1: 120,000                       | 1,333.0 | 1: 60,000                  | 400.0  |
| Cyclohexyl       | 1: 35,000                | 438.0   | 1: 32,500                        | 361.0   | 1: 40,000                  | 250.0  |
| n-Heptyl         | 1: 120,000               | 1,500.0 | 1: 200,000                       | 2,222.0 | 1: 80,000                  | 533.0  |
| n-Octyl          | 1: 140,000               | 1,750.0 | <1: 25,000                       | >312.0  | <1: 25,000                 | >167.0 |
| Phenol (control) | 1:70-80                  | 1.0     | 1:80-90                          | 1.0     | 1:140-160                  | 1.0    |

While the cocci show greater resistance to the unsubstituted parachlorophenol and its lower homologues than the typhoid colon organisms (as evidenced by the higher concentrations required to kill the former), the conditions are reversed with the further increase of the molecular weight; now the coccidal efficiency increases until in the case of the n-heptyl derivative, the three cocci are killed in extremely low concentrations which, like the corresponding phenol coefficients, belong to an order of magnitude much higher than that of the preceding table. While the n-octyl p-chlorophenol is more effective against *Staph. aureus* than the n-heptyl derivative, its potency is less than that of the latter with respect to the other two cocci of Table II. It is to be noted especially that the greatest coccidal potency is shown by those derivatives which exert little or no effect upon the four organisms of the colon typhoid group previously referred to (Table I).

TABLE III  
THE BACTERICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROCHENOL UPON ACID-FAST BACTERIA.  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (1) AND PHENOL COEFFICIENTS (II)

| MINIMUM CONCENTRATIONS OF VARIOUS ANTIBIOTICS IN VARIOUS MEDIA |                                         |         |                                       |         |                                   |           |                                   |         |                            |         |
|----------------------------------------------------------------|-----------------------------------------|---------|---------------------------------------|---------|-----------------------------------|-----------|-----------------------------------|---------|----------------------------|---------|
|                                                                | MYCOBACTERIUM<br>TUBERCULOSIS (HOMINIS) |         | MYCOBACTERIUM<br>TUBERCULOSIS (AVIUM) |         | MYCOBACTERIUM<br>LEPRAE (HOMINIS) |           | MYCOBACTERIUM<br>LEPRAE (MURRIUM) |         | MYCOBACTERIUM<br>SMEGMATIS |         |
|                                                                | I                                       | II      | I                                     | II      | I                                 | II        | I                                 | II      | I                          | II      |
| p-Chlorophenol                                                 | 1: 350                                  | 3.9     | 1: 400                                | 4.0     | 1: 400                            | 4.4       | 1: 350                            | 3.9     | 1: 350                     | 3.9     |
| Alkyl Radical                                                  |                                         |         |                                       |         |                                   |           |                                   |         |                            |         |
| Methyl                                                         | 1: 1,000                                | 11.1    | 1: 1,000                              | 11.1    | 1: 1,000                          | 11.1      | 1: 900                            | 10.0    | 1: 1,200                   | 13.3    |
| Ethyl                                                          | 1: 2,500                                | 27.8    | 1: 3,000                              | 30.0    | 1: 2,000                          | 22.2      | 1: 2,000                          | 20.0    | 1: 2,300                   | 25.0    |
| n-Propyl                                                       | 1: 6,000                                | 66.7    | 1: 7,000                              | 70.0    | 1: 6,000                          | 66.7      | 1: 6,000                          | 66.7    | 1: 8,000                   | 88.9    |
| n-Butyl                                                        | 1: 16,000                               | 178.0   | 1: 14,000                             | 156.0   | 1: 16,000                         | 178.0     | 1: 20,000                         | 200.0   | 1: 14,000                  | 156.0   |
| n-Amyl                                                         | 1: 35,000                               | 389.0   | 1: 80,000                             | 333.0   | 1: 35,000                         | 350.0     | 1: 40,000                         | 400.0   | 1: 40,000                  | 400.0   |
| Sec. Amyl                                                      | 1: 20,000                               | 222.0   | 1: 25,000                             | 277.0   | 1: 25,000                         | 278.0     | 1: 30,000                         | 333.0   | 1: 25,000                  | 278.0   |
| n-Hexyl                                                        | 1: 30,000                               | 333.0   | 1: 35,000                             | 380.0   | 1: 60,000                         | 600.0     | 1: 70,000                         | 700.0   | 1: 100,000                 | 1,111.0 |
| Cyclohexyl                                                     | 1: 25,000                               | 278.0   | 1: 25,000                             | 278.0   | 1: 25,000                         | 278.0     | 1: 30,000                         | 333.0   | 1: 25,000                  | 278.0   |
| n-Heptyl                                                       | < 1: 40,000                             | > 400.0 | < 1: 60,000                           | > 600.0 | < 1: 100,000                      | > 1,000.0 | < 1: 40,000                       | > 400.0 | 1: 100,000                 | 1,250.0 |
| Phenol (control)                                               | 1: 90-100                               | 1.0     | 1: 90-100                             | 1.0     | 1: 90-100                         | 1.0       | 1: 90-100                         | 1.0     | 1: 90-100                  | 1.0     |

The susceptibility of the acid-fast bacteria (Table III) to the action of the monoalkyl homologues of parachlorophenol resembles that of the cocci in Table II both in a qualitative and a quantitative sense. The extraordinarily great bactericidal efficacy of the higher homologues against the organisms of the acid-fast group is particularly noteworthy in view of their reputed resistance to the action of phenolic disinfectants.

The "quasi-specific" feature of the germicidal action of the higher homologous orthoalkyl parachlorophenols has a parallel in the differentiation of

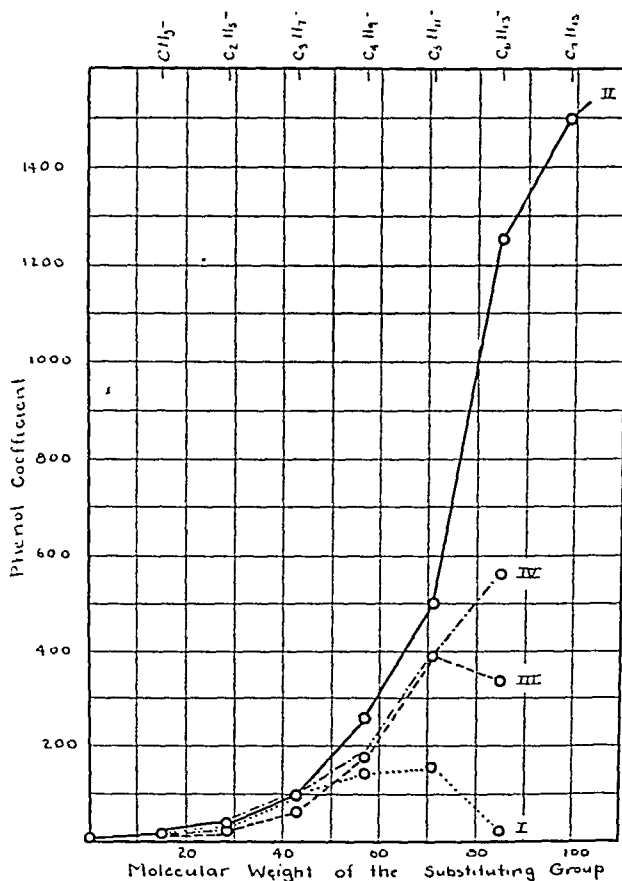


Chart 1.—The germicidal action of orthoalkyl derivatives of parachlorophenol. Test organisms: I, *Eberthella typhi*; II, *Staphylococcus aureus*; III, *Mycobacterium tuberculosis* (hom.); IV, *Monilia albicans*.

bacteria by means of the Gram stain and also in the action of certain basic dyes upon bacteria.<sup>5, 6</sup> More particularly, it appears that a gram-positive character and a susceptibility to the homologues with a higher molecular weight, run parallel in most but not in all cases. Thus *Micrococcus catarrhalis* forms an exception, in that in spite of its negative Gram reaction, it behaves much like the gram-positive organisms in this respect. However, it shows a similar behavior toward certain dyes. By way of advance information, it may be stated that *Vibrio cholerae* reacts essentially in the same manner to the higher homologues of this and other series of phenol derivatives, in spite

of its gram-negative character. Thus, while there are several examples of gram-negative microorganisms behaving like those of a positive Gram character in regard to their selective susceptibility to the "quasi-specific" action of the higher homologues of "phenolic" compounds, we do not know, to date, of any case of a gram-positive microorganism behaving under these conditions like the gram-negative bacteria of the typhoid colon group.

In spite of the entirely different morphologic character of the pathogenic fungi dealt with in Table IV, one is confronted again by the regularity in the functional reaction between the chemical constitution and fungicidal action, and by the somewhat astonishing qualitative and quantitative resemblance of this relation with that observed in the case of the cocci in Table II and of the acid-fast bacteria in Table III. Considered by itself, Table IV reveals the presence of several extremely effective fungicides in this series of compounds.

TABLE IV

THE FUNGICIDAL ACTION OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL.  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

|                  | ACHORION<br>SCHÖNLEINII |       | TRICHOPHYTON<br>ROSACEUM |       | MONILIA<br>ALBICANS |         |
|------------------|-------------------------|-------|--------------------------|-------|---------------------|---------|
|                  |                         |       |                          |       |                     |         |
|                  | I                       | II    | I                        | II    | I                   | II      |
| p-Chlorophenol   | 1: 300                  | 3.3   | 1: 500                   | 4.2   | 1: 400              | 4.0     |
| Alkyl Group      |                         |       |                          |       |                     |         |
| Methyl           | 1: 500                  | 7.1   | 1: 1,400                 | 11.7  | 1: 1,000            | 11.1    |
| Ethyl            | 1: 2,250                | 25.0  | 1: 2,750                 | 27.5  | 1: 3,250            | 32.5    |
| n-Propyl         | 1: 5,000                | 714.0 | 1: 10,000                | 83.3  | 1: 10,000           | 100.0   |
| n-Butyl          | 1: 14,000               | 156.0 | 1: 16,000                | 160.0 | 1: 16,000           | 178.0   |
| n-Amyl           | 1: 25,000               | 278.0 | 1: 40,000                | 400.0 | 1: 35,000           | 389.0   |
| Sec. Amyl        | 1: 16,000               | 229.0 | 1: 30,000                | 250.0 | 1: 20,000           | 182.0   |
| n-Hexyl          | 1: 25,000               | 357.0 | 1: 50,000                | 500.0 | 1: 50,000           | 556.0   |
| Cyclohexyl       | 1: 20,000               | 222.0 | 1: 50,000                | 417.0 | 1: 30,000           | 300.0   |
| n-Heptyl         | 1: 14,000               | 175.0 | 1: 60,000                | 667.0 | < 1: 40,000         | > 363.0 |
| Phenol (control) | 1: 70-90                | 1.0   | 1: 90-110                | 1.0   | 1: 90-110           | 1.0     |

Another illustration of the existence of a selective, "quasi-specific" effect is offered by the graphic presentation in Chart 1 in which a set of data obtained with one representative organism selected from each of the Tables I to IV has been used, viz., *Eberthella typhi*, *Staph. aureus*, *Mycobact. tuberculosis* (hom.), and *Monilia albicans*. This chart also illuminates the difference in the order of magnitude of the bactericidal potencies with respect to the several groups of microorganisms under discussion.

#### POLYALKYL DERIVATIVES

As Table V indicates, this group of compounds (listed in the order of increasing molecular weight) also comprises some highly effective microbicides. The relation between the chemical constitution and germicidal action, while being distinctly in evidence, is not of a sufficiently direct character to permit graphic presentation. Here, too, as in the monoalkyl series, the weight of the substituting groups determines the selective, "quasi-specific" action.

TABLE V  
THE GERMICIDAL ACTION OF POLYALKYL DERIVATIVES OF PARACHLOROPHENOL.  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

| DERIVATIVES OF 4-CHLORO-<br>PHENOL | BACTERIELLA<br>TYPHI |        | STAPHYLOCOCCUS<br>AUREUS |         | STREPTOCOCCUS<br>(HEMOL. STRAIN) |         | MYCOBACTERIUM<br>SMERGMATIS |        | TRICHOPHYTON<br>ROSACEUM |         |
|------------------------------------|----------------------|--------|--------------------------|---------|----------------------------------|---------|-----------------------------|--------|--------------------------|---------|
|                                    | I                    | II     | I                        | II      | I                                | II      | I                           | II     | I                        | II      |
| 3-Methyl-<br>3, 5-Dimethyl-        | 1: 1,500             | 10.7   | 1: 900                   | 11.3    | 1: 900                           | 11.3    | 1: 1,000                    | 11.1   | 1: 1,200                 | 11.0    |
| 6-Ethyl-3-methyl-                  | 1: 4,500             | 30.0   | 1: 1,800                 | 25.7    | 1: 2,750                         | 27.5    | 1: 2,250                    | 28.1   | 1: 2,500                 | 25.0    |
| 6-Ethyl-3-methyl-                  | 1: 9,000             | 64.3   | 1: 4,000                 | 50.0    | 1: 5,000                         | 55.6    | 1: 5,000                    | 55.6   | 1: 6,000                 | 60.0    |
| 6-n-Propyl-3-methyl-               | 1: 20,000            | 133.0  | 1: 16,000                | 200.0   | 1: 16,000                        | 178.0   | 1: 14,000                   | 156.0  | 1: 15,000                | 150.0   |
| 6-iso-Propyl-3-methyl-             | 1: 16,000            | 107.0  | 1: 12,000                | 150.0   | 1: 11,000                        | 138.0   | 1: 11,000                   | 138.0  | 1: 14,000                | 140.0   |
| 2-Ethyl-3, 5-dimethyl-             | 1: 7,500             | 46.4   | 1: 8,500                 | 106.0   | 1: 8,500                         | 94.4    | 1: 11,000                   | 122.0  | 1: 13,000                | 130.0   |
| 6-sec. Butyl-3-methyl-             | 1: 7,500             | 50.0   | 1: 40,000                | 500.0   | 1: 32,000                        | 361.0   | 1: 35,000                   | 389.0  | 1: 40,000                | 364.0   |
| 2-iso-Propyl-3, 5-dimethyl-        | 1: 13,000            | 81.3   | 1: 25,000                | 313.0   | 1: 25,000                        | 313.0   | 1: 32,500                   | 325.0  | 1: 27,500                | 275.0   |
| 6-Diethylmethyl-3-methyl-          | 1: 3,500             | 23.3   | 1: 50,000                | 625.0   | 1: 55,000                        | 611.0   | 1: 70,000                   | 777.0  | 1: 50,000                | 455.0   |
| 6-iso-Propyl-2-ethyl-3-methyl-     | 1: 8,500             | 56.7   | 1: 16,000                | 200.0   | 1: 14,000                        | 175.0   | 1: 18,000                   | 200.0  | 1: 16,000                | 145.0   |
| 2-sec. Butyl-3, 5-dimethyl-        | 1: 4,000             | 28.6   | 1: 45,000                | 563.0   | 1: 50,000                        | 556.0   | 1: 50,000                   | 556.0  | 1: 60,000                | 545.0   |
| 2-sec. Amyl-3, 5-dimethyl-         | (1: 2,500)           | (15.6) | 1: 60,000                | 750.0   | 1: 80,000                        | 889.0   | 1: 70,000                   | 700.0  | < 1: 40,000              | > 400.0 |
| 2-Diethylmethyl-3, 5-dimethyl-     | > 1: 2,000           | < 13.0 | 1: 80,000                | 1,143.0 | 1: 80,000                        | 1,000.0 | 1: 60,000                   | 667.0  | 1: 70,000                | 700.0   |
| 6-sec. Octyl-3-methyl-             | (1: 3,000)           | (21.4) | < 1: 8,000               | > 89.0  | 1: 11,000                        | 122.0   | < 1: 7,000                  | > 70.0 | > 1: 8,000               | < 72.0  |
| Phenol (control)                   | 1: 140-150           | 1.0    | 1: 70-80                 | 1.0     | 1: 80-90                         | 1.0     | 1: 90-100                   | 1.0    | 1: 90-110                | 1.0     |



As to the effect upon the germicidal action, of the distribution of the added weight over several substituting radicals, a comparison, e.g., of the substituted parachlorophenol derivatives with a total of four substituting carbon atoms will furnish the necessary illustration. It shows that substitution of parachlorophenol by one alkyl group generally leads to a more effective compound than substitution by two or three alkyl groups with the same total number of carbon atoms; similarly, dialkyl substituted derivatives are considerably more germicidal than trialkyl substituted ones.

It is found in this group, too, that isoalkyl substituted compounds are less effective than the corresponding normal alkyl derivatives. This follows from the comparison of 6-n-propyl-3-methyl-4-chlorophenol and 6-isopropyl-3-methyl-4-chlorophenol (chlorothymol); thus the n-propyl derivative is distinctly more potent than chlorothymol.

Polyalkyl compounds of equal molecular weight, containing isoalkyl groups may show considerable variation in their germicidal action, depending upon their structure.

Whereas in the case of the normal monoalkyl derivatives the maximum effect upon *Eberthella typhi* was shown by a compound with five carbon atoms in the side chain, in the case of the polyalkyl derivatives this maximum is reached by a compound with a total of four substituting carbon atoms. As to the other test organisms in Table V the derivatives with a total of seven carbon atoms generally show the greatest microbicidal efficacy.

#### AROMATIC DERIVATIVES

The aromatic derivatives of parachlorophenol prepared and studied to date comprise the benzyl, and phenylethyl derivatives of parachlorophenol and its methyl homologues listed in Table VI. Because of the greater weight of the aromatic radicals, such substitution might be expected to produce compounds which in their antibacterial behavior would resemble the higher alkyl homologues, i.e., manifest a selective or "quasi-specific" action upon gram-positive and those other organisms which show an analogous behavior in this regard. In keeping with the general idea that particularly the molecular weight determines the point at which the "quasi-specific" germicidal effect becomes apparent, it is found that actually the compounds of this group show a selective action in the above sense, as evidenced by the numerical values of their germicidal potency.

There are among the aromatic derivatives some highly potent germicides. As to the effect of the chemical constitution upon germicidal action, it is noteworthy that the step from the benzyl derivative to the next one with a higher molecular weight is accompanied by different results depending upon the place in the molecular structure to which the additional weight is attached. Thus, 5-methyl-2-benzyl-4-chlorophenol is much less effective than 2-benzyl-4-chlorophenol against the four test organisms of the typhoid-colon group, while 2-phenylethyl-4-chlorophenol is very much more effective than the former. With reference to the other organisms, the difference in the germicidal potencies of the methyl-benzyl and the phenylethyl derivatives is less pronounced.

TABLE VI  
THE GERMICIDAL ACTION OF AROMATIC DERIVATIVES OF PARACHLOROPHENOL.  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

| DERIVATIVES OF<br>4-CHLOROPHENOL | EBERTHIELLA TYPHI |        | EBERTHIELLA PARADYSEN-<br>TERIAE |      | SALMONELLA SCHOTTMÜLLERI<br>(B. PARATYPHIOSUS B) |        | ESCHERICHIA COLI |        |
|----------------------------------|-------------------|--------|----------------------------------|------|--------------------------------------------------|--------|------------------|--------|
|                                  | I                 | II     | I                                | II   | I                                                | II     | I                | II     |
| 2-Benzyl-                        | 1:10,000          | 71.4   | 1:12,000                         | 92.3 | 1:10,000                                         | 71.4   | 1:10,000         | 71.4   |
| 5-Methyl-2-benzyl-               | 1: 2,750          | 18.3   | 1: 4,500                         | 34.6 | 1: 4,000                                         | 28.6   | (1: 2,500)       | (19.2) |
| 2-Phenylethyl-                   | 1:16,000          | 100.0  | 1:12,000                         | 80.0 | 1:10,000                                         | 71.4   | 1: 5,000         | 35.7   |
| 3, 5-Dimethyl-2-benzyl-          | (1: 4,500)        | (32.1) | 1: 5,000                         | 33.3 | (1: 3,000)                                       | (21.4) | (1: 2,500)       | (17.9) |
| Phenol (control)                 | 1:140-150         | 1.0    | 1:140-160                        | 1.0  | 1:140-150                                        | 1.0    | 1:140-150        | 1.0    |

| DERIVATIVES OF<br>4-CHLOROPHENOL | STAPHYLOCOCCUS<br>AUREUS |     | STREPTOCOCCUS<br>(HEMOL. STRAIN) |     | MYCOBACTERIUM<br>TUBERCULOSIS<br>(HOM.) |            | MYCOBACTERIUM<br>LEPRAE (MUR.) |     | MYCOBACTERIUM<br>SMEGMATIS |     | TRICHOPHYTON<br>ROSACEUM |     |
|----------------------------------|--------------------------|-----|----------------------------------|-----|-----------------------------------------|------------|--------------------------------|-----|----------------------------|-----|--------------------------|-----|
|                                  | I                        | II  | I                                | II  | I                                       | II         | I                              | II  | I                          | II  | I                        | II  |
| 2-Benzyl-                        | 1:16,000                 | 200 | 1:18,000                         | 225 | 1:16,000                                | 178        | 1:20,000                       | 222 | 1:20,000                   | 222 | 1:30,000                 | 273 |
| 5-Methyl-2-benzyl-               | 1:30,000                 | 375 | 1:35,000                         | 389 | indefinite                              | indefinite | indefinite                     | 278 | 1:25,000                   | 278 | 1:40,000                 | 400 |
| 2-Phenylethyl-                   | 1:30,000                 | 375 | 1:40,000                         | 500 | 1:30,000                                | 333        | 1:30,000                       | 333 | 1:37,500                   | 375 | 1:37,500                 | 375 |
| 3, 5-Dimethyl-2-benzyl-          | 1:60,000                 | 750 | 1:70,000                         | 778 | indefinite                              | indefinite | indefinite                     | 333 | 1:30,000                   | 333 | 1:70,000                 | 636 |
| 3-Methyl-6-phenylethyl-          | 1:30,000                 | 375 | 1:25,000                         | 250 | 1:16,000                                | 178        | 1:20,000                       | 222 | 1:20,000                   | 222 | 1:40,000                 | 364 |
| Phenol (control)                 | 1:70-80                  | 1   | 1:80-90                          | 1   | 1:90-100                                | 1          | 1:90-100                       | 1   | 1:90-100                   | 1   | 1:100-110                | 1   |

A further increase in the molecular weight lowers the germicidal action with reference to the bacteria of the typhoid-colon group as shown by the figures obtained with 3, 5-dimethyl-2-benzyl-4-chlorophenol. However, the germicidal potency is increased considerably with regard to the other four organisms. Curiously, 3-methyl-6-phenylethyl-4-chlorophenol, which is isomeric with 3,5-dimethyl-2-benzyl-4-chlorophenol, is not only less effective than the latter, but also, in most instances, weaker than the 2-phenyl-ethyl derivative.

#### BACTERIOSTATIC ACTION

In addition to being potent germicides, certain substituted chlorophenol derivatives manifest considerable inhibitory capacity as well. An investigation of the bacteriostatic effect upon *Staphylococcus aureus* and upon three mycobacteria, viz., smegmatis, tuberculosis (hom.), and leprae (hom.) was carried out with 2-n-amyl-4-chlorophenol and 2-n-heptyl-4-chlorophenol. This experiment was conducted in such a manner as to permit the determination not only of the minimum concentrations required to prevent the growth of the microorganisms, but also that of the status of their viability during the course of their exposure to the inhibitory concentrations. This was accomplished by making transfers from the "inhibitory" media into fresh media after different periods of time.

The results obtained with the two compounds and the four organisms mentioned above are given in Table VII. It is to be noted among other things that whereas in the case of the 2-n-amyl-4-chlorophenol the minimum germicidal concentration is practically four times the minimum inhibitory one, in the case of 2-n-heptyl-4-chlorophenol it is only twice the latter (in the two cases where such comparison can be made).

The effect of  $P_H$  of the medium upon the inhibition by 2-n-amyl-4-chlorophenol and 2-cyclohexyl-4-chlorophenol of the growth of *Staph. aureus* and of *Mycobact. tuberculosis (hom.)* is illustrated in Table VIII. It is found that variations of  $P_H$  in both directions (i.e., to the acid and the alkaline sides) have little effect upon the bacteriostatic capacity of these compounds, a result of distinct practical significance.

#### TOXICITY

It is natural that the discovery of compounds showing such intensive antibacterial properties, should suggest the extension of this study beyond the stage of "in vitro" experiments, and encourage the inquiry into their availability for chemotherapeutic purposes. Some phases of this work are being planned at this time, others are under way.

One important condition of the potential usefulness of a drug for such purposes is a low organotropic against a high parasitotropic action. A preliminary series of experiments aiming at the determination of toxicity of the series of alkyl parachlorophenol derivatives (upon subcutaneous injection in mice\*) shows that this toxicity decreases with increasing molecular weight

\*For the purpose of this test the compounds in question were emulsified in a 0.5 per cent traccanth solution, the proportion of the total quantity of fluid injected to the weight of the animal being approximately the same in all cases.

TABLE VII  
BACTERIOSTATIC ACTION  
A--2-N-AMYL-4-CHLOROPHENOL  
B--2-N-HEPTYL-4-CHLOROPHENOL

| MICROORGANISM                               | COM-<br>POUND | TIME OF<br>OBSERVA-<br>TION IN<br>HOURS | MINIMUM<br>INHIBITORY<br>CONCEN-<br>TRATIONS | NO GROWTH IN SUBCULTURES FROM THE FOLLOWING CONCENTRATIONS AFTER |           |           |           |           |           |           |           |           |  |
|---------------------------------------------|---------------|-----------------------------------------|----------------------------------------------|------------------------------------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|--|
|                                             |               |                                         |                                              | 15 MIN.                                                          | 1 HOUR    | 2 HOURS   | 4 HOURS   | 6 HOURS   | 24 HOURS  | 48 HOURS  | 72 HOURS  |           |  |
|                                             |               |                                         |                                              |                                                                  |           |           |           |           |           |           |           |           |  |
| <i>Staphylococcus aureus</i>                | A             | 96                                      | 1:150,000                                    | 1:40,000                                                         | 1:50,000  | 1:60,000  | 1:60,000  | 1:80,000  | 1:120,000 | 1:150,000 | 1:150,000 | 1:150,000 |  |
|                                             | B             |                                         | 1:200,000                                    | 1:120,000                                                        | 1:150,000 | 1:150,000 | 1:150,000 | 1:200,000 | 1:200,000 | 1:200,000 |           |           |  |
| <i>Mycobacterium smegmatis</i>              | A             | 120                                     | 1:200,000                                    | 1:50,000                                                         | 1:80,000  | 1:120,000 | 1:120,000 | 1:120,000 | 1:150,000 | 1:150,000 | 1:200,000 | 1:200,000 |  |
|                                             | B             |                                         | 1:200,000                                    | 1:120,000                                                        | 1:150,000 | 1:150,000 | 1:150,000 | 1:200,000 | 1:200,000 | 1:200,000 |           |           |  |
| <i>Mycobacterium tuberculosis (hominis)</i> | A             | 168                                     | 1:150,000                                    | 1:30,000                                                         | 1:40,000  | 1:50,000  | 1:50,000  | 1:60,000  | 1:150,000 | 1:150,000 | 1:150,000 | 1:150,000 |  |
|                                             | B             |                                         | 1:200,000                                    | 1:80,000                                                         | 1:80,000  | 1:100,000 | 1:150,000 | 1:150,000 | 1:200,000 | 1:200,000 | 1:200,000 |           |  |
| <i>Mycobacterium leprae (hominis)</i>       | A             | 168                                     | 1:150,000                                    | 1:40,000                                                         | 1:50,000  | 1:50,000  | 1:50,000  | 1:60,000  | 1:100,000 | 1:150,000 | 1:150,000 | 1:150,000 |  |
|                                             | B             |                                         | 1:200,000                                    | 1:80,000                                                         | 1:100,000 | 1:100,000 | 1:100,000 | 1:120,000 | 1:150,000 | 1:200,000 | 1:200,000 |           |  |

(Table IX); in other words increasing antibacterial potency (with reference to most of the test organisms) is accompanied by decreasing toxicity. Thus in the case of several of the higher homologues, the enormous dose of 20 mg. per gm. of body weight (corresponding to about 1.5 kg. for an adult human being of 150 pounds) is tolerated. Even though it is inadmissible to draw direct conclusions as to the probable pharmacologic effect upon one species from the observed effect upon another, the preliminary toxicity experiments, too, indicate that the extension of this inquiry into the therapeutic field is not without some promise of success.

#### EXPERIMENTAL PART

*Determination of Bactericidal Action.*—All tests were carried out at 37° C. In all cases 0.5 c.c. of culture was added to 5 c.c. of the various dilutions of the antiseptic substances.

As to the test organisms of the typhoid colon group, cultures of *Eberthella typhi* were procured from the Bacteriological Laboratory of the Food and Drug Administration of the U. S. Department of Agriculture, those of *Eberthella paratyphenteriae*, *Salmonella schottmülleri*, and *Escherichia coli* from the American Type Culture Collection. The method of culturing and preparing these organisms for the tests followed closely that described by Ruehle and Brewer<sup>7</sup> in the case of *Eberthella typhi*, and the testing technic used was the same as given in detail by these authors, i.e., transfers from the medication tubes into fresh media were made after exposures of five, ten, and fifteen minutes, respectively, and the readings were taken after forty-eight hours' incubation. For the sake of space economy the results of the five- and fifteen-minute exposures are left out in Table I (and incidentally also in Tables II to VI), and those of the ten-minute exposures only are given. The "phenol coefficients" were calculated in all cases from the results of the ten-minute exposures to the compounds under discussion on one hand, and to phenol (carbolic acid) on the other. A phenol control test always accompanied the tests with these compounds, carried out on any given day.

As to the three species of pathogenic cocci, the stock culture of *Staphylococcus aureus* was obtained from the Bacteriological Laboratory of the Food and Drug Administration, those of streptococcus and of *Micrococcus catarrhalis* from the Lederle Laboratories. *Staphylococcus aureus* was grown and applied in the test according to the directions given in Ruehle's and Brewer's publication, previously referred to. The same methods were applied in the case of *Micrococcus catarrhalis* yielding results of very satisfactory regularity.

The hemolytic strain of streptococcus used was one of considerable resistance, approaching that of *Staphylococcus aureus*, as evidenced by the results of the phenol-control tests. According to information received from Mr. E. F. Voight, bacteriologist of the Lederle Laboratories, whose assistance is gratefully acknowledged herewith, this strain was isolated originally from a case of scarlet fever and was selected as a scarlet fever type by Dr. A. R. Dochez at Johns Hopkins University. The stock culture for the test was grown on beef infusion peptone broth adjusted to P<sub>H</sub> 7.2-7.4 and was used when twenty-

four hours old. After exposure to the various dilutions of the antiseptics, transfers were made into beef extract peptone broth containing 0.5 per cent of glucose and adjusted to  $P_H$  7.2-7.4.

In the group of the acid-fast bacteria the strains of human and avian tuberculosis and those of human and rat leprosy were procured (with several others, not yet utilized in this investigation) from the U. S. Marine Hospital in Carville, Louisiana, through the courtesy of Dr. O. E. Denney to whom the authors wish to express their gratitude at this opportunity. The four original culture tubes received, were described as follows:

- M. tuberculosis hominis* Koch
- M. tuberculosis avium* Arlong (Phipps)
- M. leprae human* Clegg 1, Duval No. 105
- M. leprae murium*, Nat. Inst. Health

These four cultures were chosen because of the rapid growth which could be detected easily twenty-four hours after seeding. The medium for both stock cultures and subcultures was beef extract peptone broth containing 5 per cent of glycerin and adjusted to  $P_H$  7.4. The stock cultures were grown for from five to seven days in strong bottles containing glass beads. Shortly before the test the bottles were stoppered with sterile rubber stoppers and shaken until a homogeneous suspension was obtained. This suspension was then poured through a sterile 200 mesh monel metal sieve in order to eliminate the larger particles. Final readings were made after incubation for seven days.

The original strain of *Mycobacterium smegmatis* was obtained from the American Type Culture Collection. It was grown and applied in the same manner as *Staphylococcus aureus* except that the stock emulsion for the test was forty-eight hours old and that final readings of the subcultures were made after one week's incubation.

As to the group of pathogenic fungi, the cultures of *Achorion schönleini* and of *Monilia albicans* were procured from the American Type Culture Collection. The authors are very grateful to Dr. E. D. Osborne of Buffalo, N. Y., for two cultures of trichophyton, one of which was used in this work.

*Achorion schönleini* and *Trichophyton rosaceum* were treated in the same manner. Both were grown on Sabouraud's agar which was also used for subcultures. The stock suspension for the test was prepared in the following manner: The growth of from ten to fifteen days was removed from the slant by means of a hooked platinum wire and placed in a sterile bottle with a layer of glass beads at the bottom. Four cubic centimeters of sterile normal saline was added, the bottle stoppered with a sterile rubber stopper and shaken vigorously, until the mass was broken up completely. Then another 6 c.c. of saline was added and the bottle shaken again. The suspension thus obtained was poured into a glass tube and the latter centrifuged for one minute at the lowest speed, in order to allow the large unbroken particles to settle to the bottom. The supernatant suspension was removed with a sterile pipette and diluted with double the amount of saline. As a rule the yield

from each culture tube was about 15 c.c. of suspension ready for use. The subcultures were held at 28° C. for ten days before the final readings were made.

*Monilia albicans* was also grown and subcultured on Sabouraud's agar. The suspension in normal saline was obtained by washing off a three- to five-day-old growth from the surface of the slant. Each tube gave 10 c.c. of the suspension for the test. Readings were made after incubation at 28° C. for five days.

#### DETERMINATION OF BACTERIOSTATIC ACTION

Regular bacteriostatic tests were carried out in the customary fashion by inoculation of media containing different concentrations of the antiseptic substances, and observation of the incubated tubes.

Where it was desired to determine the status of viability of the microorganisms in the presence of bacteriostatic concentrations, in relation to the time of exposure, the following procedure was used: To 9 c.c. of broth (of the same composition as used in the bactericidal tests) 1 c.c. of the antiseptic solution was added, containing ten times the concentration which appears in Tables VIII and IX. A spiral loop (of five turns of 2.18 mm. inside diameter, wire gage 23B and S., and holding approximately 0.02 c.c.) was used to plant the culture in this mixture previously warmed to 37° C. The tubes were kept

TABLE VIII

THE EFFECT OF  $P_H$  UPON THE BACTERIOSTATIC POTENCY. MINIMUM BACTERIOSTATIC CONCENTRATIONS IN FORTY-EIGHT HOURS AT 37° C.

|                             | STAPHYLOCOCCUS AUREUS |           |           | MYCOBACTERIUM TUBERCULOSIS (HUMAN) |           |           |
|-----------------------------|-----------------------|-----------|-----------|------------------------------------|-----------|-----------|
|                             | $P_H$ 6.0             | $P_H$ 6.8 | $P_H$ 7.6 | $P_H$ 6.6                          | $P_H$ 7.4 | $P_H$ 8.2 |
| 2-n-Amyl-4-chlorophenol     | 1:200,000             | 1:200,000 | 1:150,000 | 1:150,000                          | 1:150,000 | 1:150,000 |
| 2-Cyclohexyl-4-chlorophenol | 1:70,000              | 1:70,000  | 1:100,000 | 1:150,000                          | 1:150,000 | 1:150,000 |

TABLE IX

TOXICITY OF ORTHOALKYL DERIVATIVES OF PARACHLOROPHENOL. SUBCUTANEOUS INJECTION IN MICE

MINIMUM LETHAL DOSES IN MILLIGRAMS PER GRAM OF BODY WEIGHT

| DERIVATIVES OF 4-CHLOROPHENOL | M. F. D. MG. PER GRAM |
|-------------------------------|-----------------------|
| p-Chlorophenol                | 0.6                   |
| Methyl                        | 2.0                   |
| Ethyl                         | 4.0                   |
| n-Propyl                      | 6.0                   |
| n-Butyl                       | 15.0                  |
| n-Amyl                        | >20.0                 |
| sec. Amyl                     | 15.0                  |
| n-Hexyl                       | >20.0                 |
| Cyclohexyl                    | >20.0                 |
| n-Heptyl                      | >20.0                 |

in the incubator for the stated periods of time and transfers were made into fresh media after fifteen minutes, one, two, four, six, twenty-four, forty-eight, and seventy-two hours with a loop of the same size. Final readings of the subcultures were made after incubation for forty-eight hours in the case of *Staphylococcus aureus*, and after one week in that of the acid-fast bacteria.

It is of possible practical interest that according to the outcome of several tests, both stock solutions and emulsions of the compounds under discussion, and also the final dilutions in the medication tubes may be sterilized in the autoclave without suffering any impairment of their bactericidal potency.

#### SUMMARY

The bactericidal and fungicidal action of the homologous series of parachlorophenol derivatives has been investigated with the aid of the following microorganisms: *Eberthella typhi*, *Escherichia coli*, *Eberthella paradysenteriae* (Flexner), *Salmonella schottmülleri*, *Staphylococcus aureus*, streptococcus (hemol. strain), *Micrococcus cutarrhalis*, *Mycobacterium tuberculosis* (hom.) and (avium), *Mycobacterium leprae* (hom.) and (mur.), *M. smegmatis*, *Monilia albicans*, *Trichophyton rosaceum* and *Achorion schönleini*.

In confirmation of previous preliminary findings (stated in the initial paragraphs of this paper), the presence in this series of some derivatives of an extraordinarily high bactericidal and fungicidal potency has been established. The relationship between the chemical constitution and antibacterial action has been discussed in some detail. A "quasi-specific" effect of certain higher alkyl homologues upon the test organisms, exclusive of the bacteria of the typhoid-colon group, appears to divide the microorganisms in two groups, the division agreeing partly, but not entirely with that obtained by means of the Gram stain, and evidently also with that based upon their susceptibility to the inhibitory action of certain basic dyes. Nevertheless, the class of compounds under consideration comprises some bactericides, highly effective against the organisms of the typhoid colon group as well.

In addition to the monoalkyl derivatives, several polyalkyl and aromatic compounds were studied and the regularities in their antibacterial behavior were pointed out.

The bacteriostatic potency of some of the derivatives was also investigated and found to exist to a remarkable degree not only with respect to staphylococcus, but also with respect to several acid-fast strains. The comparative independence of the bacteriostatic capacity from the  $P_H$  within rather wide limits was emphasized as being of possible practical significance.

This great antibacterial efficacy of certain derivatives of the homologous parachlorophenol series was found to be accompanied by very low toxicity to mice. Specifically, the increase in weight of the substituting alkyl radical which causes, within certain limits, an increase in the germicidal potency, produces also a sharp decline in the toxicity to animals. This combination of high antibacterial capacity and low toxicity to animals, as encountered in several instances, suggests the extension of the present inquiry into the field of chemotherapy.

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## NORMAL ERYTHROCYTE, HEMOGLOBIN AND PACKED CELL VOLUME STANDARDS IN YOUNG MEN\*

### A STUDY OF 100 SUBJECTS

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THE unsatisfactory character of the data on which the long-accepted normal erythrocyte and hemoglobin values are based has been pointed out frequently in recent years. The increasing use of the blood indices or of the average volume and hemoglobin content of the individual erythrocyte in differentiating anemias has emphasized the need of dependable normal values. In an effort to provide adequate data for reliable standards, a number of extensive studies have been made on young men. Haden<sup>10</sup> in 1922 published a study which included observations on 20 men between the ages of eighteen and thirty. This series was later increased to 35.<sup>14</sup> Osgood<sup>21</sup> in 1926 followed with data on the blood of 137 men between nineteen and thirty. Wintrobe and Miller<sup>33</sup> made similar observations on 100 men between the ages of twenty and thirty. Foster and Johnson<sup>7</sup> followed with a similar study of 115 normal men. Even with these data, general erythrocyte and hemoglobin standards are by no means established,<sup>1, 5</sup> and the need for additional study in other localities has often been pointed out.<sup>12, 24, 26</sup>

The present study is based upon the observations made on 100 men between the ages of twenty and thirty. The subjects were medical and college students, all of whom had been found in good health by thorough physical examination. Since previous investigation has shown that both muscular activity<sup>17</sup> and rest<sup>22</sup> produce significant fluctuations in the erythrocyte count, the samples of blood were drawn as soon as the subjects presented themselves at the laboratory.

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No other effort was made to control physical activity. The data are, therefore, believed to be comparable to previous studies in which the subjects have, also, apparently been chosen at random with respect to physical activity. Most of the observations were made during the month of September.

#### METHODS

A 6 c.c. quantity of blood was drawn from an arm vein, 5 c.c. being placed in a 15 c.c. graduated centrifuge tube containing 1 c.c. of 1.6 per cent sodium oxalate solution. The 1 c.c. of blood was placed in a second tube containing a small amount of dry oxalate and was later used for making counts and hemoglobin determinations. All tubes were tightly stoppered immediately, and samples were centrifuged or diluted before any known changes which would affect any of the observations could occur in the blood. Precautions were taken to prevent stasis in arm veins when withdrawing blood.

Cell volume was ascertained by centrifuging the tubes containing the 5 c.c. of blood in 1.6 per cent oxalate for one hour, which is considered adequate by Haden,<sup>13</sup> at a speed of 2,800 r.p.m. in a centrifuge with a working radius of 13.5 cm. The tubes were allowed to return to room temperature before readings were made. The percentage of cells was calculated from the readings of the graduated scale on the tube.

Erythrocyte counts were made after dilution in Trenner pipettes, in Levy-Hausser chambers having improved Neubauer ruling, all of which apparatus had been tested by the U. S. Bureau of Standards. The pipettes were agitated for three minutes in a mechanical shaker which was carefully checked to eliminate the possibility of any mechanical hemolysis. At least two dilutions with Hayem's solution were made from each sample and, if necessary, additional dilutions were made until counts from separate pipettes were obtained which agreed within 100,000 cells. Blood was thoroughly mixed by repeated inversion of tubes before samples were taken out, and diluted samples were allowed to settle completely in the chamber before counting. Trenner pipettes were checked with U. S. Standard Thoma pipettes and were found to give concordant readings.

Hemoglobin was estimated by the Newcomer method. Determinations were made after the acid hematin solution had stood for one hour. All readings were made by daylight. Each reading recorded is the average of six observations of the colorimeter. In order to verify the dependability of the

TABLE I

| BLOOD SAMPLE<br>NUMBER | NEWCOMER<br>METHOD | OXYGEN CAPY.<br>METHOD | VARIATION OF<br>NEWCOMER |
|------------------------|--------------------|------------------------|--------------------------|
| 1                      | 10.86              | 10.84                  | +0.02                    |
| 2                      | 11.48              | 10.89                  | +0.59                    |
| 3                      | 11.83              | 11.86                  | +0.03                    |
| 4                      | 13.35              | 13.34                  | +0.01                    |
| 5                      | 14.84              | 14.52                  | +0.32                    |
| 6                      | 15.23              | 15.24                  | -0.01                    |
| 7                      | 15.15              | 15.30                  | -0.15                    |
| 8                      | 16.12              | 15.79                  | +0.33                    |
| 9                      | 15.20              | 15.85                  | -0.65                    |
| 10                     | 25.78              | 25.81                  | -0.03                    |

Newcomer disc, the apparatus was checked against the Van Slyke oxygen capacity method on 10 different samples of blood. The results were as shown in Table I.

Because of the close correspondence of this series of comparative determinations, no corrections were applied to the Newcomer readings. All cell counts and hemoglobin determinations were made by the same individual.

#### THE ERYTHROCYTE COUNT

Other investigators<sup>24, 33</sup> have reviewed the literature dealing with normal erythrocyte values and have shown how prevailing standards originated from a surprisingly small number of observations. In the United States, four important studies of normal men in the age range from eighteen to thirty, and using accurate methods for erythrocyte count, total hemoglobin and total cell volume have appeared recently.

Working in Kansas, Haden observed in a series of 20 men, aged eighteen to thirty, a mean erythrocyte count of 5.08. The lowest count recorded was

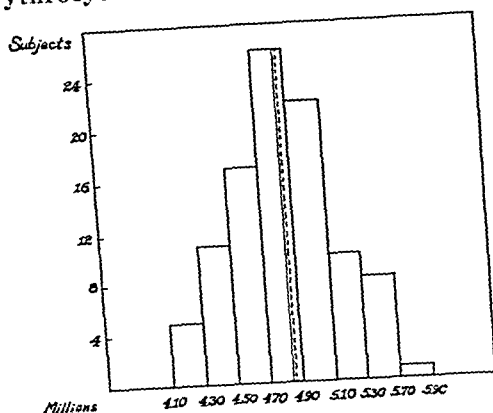


Fig. 1.—Frequency distribution of erythrocyte count in 100 young men. The median, 4.86, is indicated by a broken line and the mean, 4.84, by a solid line.

4.52 and the highest, 5.62. When the series was increased to 35 subjects, the mean count was 5.05 million. Osgood, in Oregon, found the mean erythrocyte count for the 137 men of his series to be 5.39 million, ranging from 4.4 to 6.4 million, with 90 per cent lying between 4.7 and 6.1 million. Wintrobe and Miller, working in Louisiana, observed a mean of 5.85 million in the 100 men examined by them, with 95 per cent between 5 and 6.90 million. The minimum and maximum values of this group were 4.68 and 7.53, respectively.

Emerson<sup>7</sup> in Baltimore reported the average count of 176 men between the ages of twenty and twenty-five as 5 million, with only 8 per cent below 5 million and 8.5 per cent above 6 million. Haden<sup>15</sup> has recently reported a series of counts made by medical students in Kansas City on their own blood, in which a mean of 4.997 was found, the average of 54 students in 1929 to 1930 being 4.85 million. Figures reported by various other investigators are given in Table II.

In the 100 men observed in the present study, the mean erythrocyte count was 4.84 million per cubic millimeter of blood, the counts ranging from 4.10 to 5.55 million. Of this number, 90 per cent lay between 4.31 and 5.35 million,

## TOTAL HEMOGLOBIN AND COLOR INDEX

Haden, using the Van Slyke method, found in his study of 35 men a mean total hemoglobin of 15.63 gm. per 100 c.c. of blood. Osgood, employing the Osgood-Haskins method of hemoglobin determination, standardized by the Van Slyke, found a mean of 15.76 gm. in his series of 137 men. Ninety per cent of the values were between 14 and 18 gm. Wintrobe and Miller,<sup>22, 24</sup> using the Newcomer hemoglobinometer corrected by the Van Slyke method, obtained a mean hemoglobin figure of 17 for 100 male subjects, with 73 per cent of the determinations ranging between 15.5 and 18.5 gm. Foster and Johnson found a mean value of 15.63 gm. per 100 c.c. for 115 men.

Several large series of normal men have been investigated by workers in other localities. The mean values found by Harrop,<sup>16</sup> Orias,<sup>21, 22</sup> Parodi,<sup>26</sup> Sokhey,<sup>30</sup> Parjono et al.,<sup>25</sup> Tenconi,<sup>31</sup> Price-Jones,<sup>28</sup> Dill,<sup>4</sup> and more recently, by Jenkins and Don,<sup>19</sup> are shown in Table III.

The familiar work of Williamson is criticized<sup>27</sup> because a standard of recrystallized hemoglobin was used. Horneffer's<sup>18</sup> mean of 16.03 gm. per 100 c.c. for 40 men has been questioned<sup>28</sup> for the same cause.

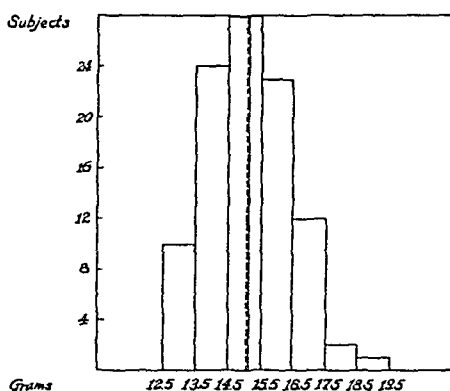


Fig. 2.—Frequency distribution of hemoglobin values in 100 young men. The median, 15.02, is indicated by a broken line and the mean, 15.12, by a solid line.

Various methods of determination have been reported. Sackett,<sup>29</sup> using the Wong iron method, found a mean value of 16.71 gm. per 100 c.c. in 15 normal men. Chia Yu Tien<sup>31a</sup> reports a series of observations on 321 healthy Chinese in which a mean of 97.4 per cent by the Sahli method was found. Komocki<sup>20</sup> records a mean of 86 per cent Sahli in 17 healthy men associated with an average red count of 5.97 million. Cabigting,<sup>2</sup> using the Bausch and Lomb hemoglobinometer, found a mean of 82.7 per cent for 22 normal male Filipinos.

The mean value found for the 100 subjects of the present study was 15.12 gm. per 100 c.c. of blood, 90 per cent of the figures lying between 13.25 and 17.03 gm. The extremes were 12.89 and 18.70 gm.

In the calculation of color index, it is of course necessary to employ an arbitrary standard for erythrocytes and hemoglobin which shall be regarded as 100 per cent. The use of 5 million is universally accepted as 100 per cent

of red cells. Osgood, on the basis of his own observations and the data of previous investigators, recommended the use of the hemoglobin coefficient, 14.7 gm., to represent 100 per cent. Wintrobe adopted 14.6 gm. as a more accurate figure to represent 100 per cent.

The inadaptability of a hemoglobin coefficient derived largely from one or two series to all localities is demonstrated by employing the figure used as 100 per cent by Wintrobe in four of the separate series being compared. The data of Osgood and of Wintrobe and Miller, when so treated, show a mean color index of 1. Haden's subjects show a mean color index of 1.07 and those of the present series, 1.08, with Wintrobe's 100 per cent figure. If, however, a hemoglobin coefficient is calculated from the combined data of Haden and of this study, the resulting figure, 15.59 gm., when used as 100 per cent hemoglobin, gives a mean color index of 1.006 for the subjects of the present series, 85 per cent of which are between 0.85 and 1.15.

#### TOTAL VOLUME OF PACKED CELLS AND VOLUME INDEX

Since the procedure followed by Osgood and by Wintrobe and Miller employed powdered oxalate as an anticoagulant, corrections were applied to

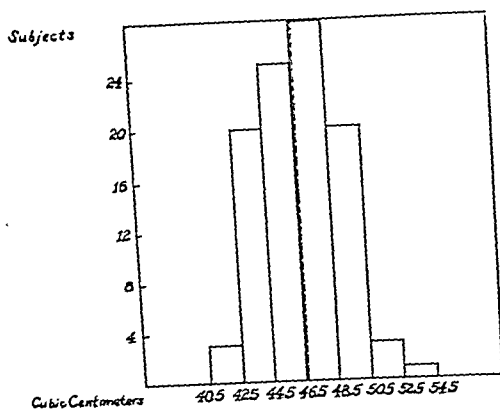


Fig. 3.—Frequency distribution of total volume of packed cells in 100 young men. The median, 46.8, is indicated by a broken line and the mean, 46.5, by a solid line.

their original figures. Osgood, using 20 mg. powdered oxalate to 10 c.c. of blood, obtained an average total volume of 44.84 c.c. of packed cells per 100 c.c. of blood in the 94 subjects who were so examined. This figure was increased by 3.5 per cent to correct for shrinkage of the cells, giving a revised figure of 46.4 c.c. packed cells per 100 c.c. of blood. Ninety per cent of the values ranged between 40 and 50 c.c.

The corrected mean of Wintrobe and Miller is 46.5 c.c. of packed cells per 100 c.c. of blood. This was obtained by adjusting for the 6.7 per cent shrinkage in volume with the 40 mg. of oxalate per 10 c.c. of blood used by them. Of the corrected values, 85 per cent ranged between 40 and 50 c.c.

The mean total cell volume found in the present series was 46.5 c.c. packed cells per 100 c.c. of blood, with 92 per cent of the total volume determinations between 43 and 50 c.c. This average agrees with Haden's first published data<sup>10</sup> in which 1.6 per cent oxalate solution was used as an anticoagulant.

Haden<sup>13</sup> has since demonstrated that 1.4 per cent oxalate is more nearly isotonic with human blood than 1.6 per cent although the difference is small. The mean value found by Foster and Johnson for 40 subjects using heparin as an anticoagulant is slightly greater, as might be expected. Peters and Van Slyke<sup>27</sup> mention the unpublished packed cell volume determinations of Eisenman as agreeing excellently with those of Haden and Osgood. Pearl and Miner<sup>26a</sup> report a mean of 45.59 c.c. in their study of 272 normal men.

In spite of the fact that the various average figures for total volume of packed cells agree so closely, the great divergence in red cell counts produces volume coefficients in each instance which are widely separated. Osgood's corrected volume coefficient is 43.04 c.c., while the high average cell count found in Wintrobe and Miller's subjects gives a volume coefficient of 39.94. Foster and Johnson calculated 41.4 as the volume coefficient of their 40 subjects. Osgood, as well as Wintrobe and Miller, employed 41 c.c. as 100 per cent in their calculations, giving volume indices of 1.00 and 0.97 respectively. The use of 41.0 c.c. as 100 per cent would give a volume index in Haden's group of 1.12 and in this series, of 1.18. Obviously, again, the standard chosen as 100 per cent must, judging from the above calculations, be a local one, at least for the present. If the data of Haden and of this series are combined, a volume coefficient of 47.4 c.c. is derived, which, when considered as 100 per cent total cell volume, gives a mean volume index in this series of 1.017 with 90 per cent between 0.89 and 1.15.

#### SATURATION INDEX

Employing the standards used by Wintrobe, a hemoglobin coefficient of 14.6 gm. and a volume coefficient of 41 c.c., the 100 subjects of Wintrobe and Miller average 1.02, the Oregon men, 0.98, and Haden's Kansas City group 0.96 in saturation index. The series under consideration would average 0.92. If, however, 15.59 gm. hemoglobin and 47.4 c.c. packed cells are taken as 100 per cent standards, the mean saturation index obtained is 0.99 in this series, with 90 per cent ranging between 0.85 and 1.12.

#### MEAN CORPUSCULAR VOLUME, HEMOGLOBIN AND HEMOGLOBIN CONCENTRATION

The method of calculating mean corpuscular volume, hemoglobin and hemoglobin concentration has been explained frequently elsewhere<sup>10, 11, 35, 36</sup> and is therefore omitted here. Mean corpuscular volume refers to the average volume of individual erythrocytes, stated in cubic micra. Mean corpuscular hemoglobin expresses the average amount of hemoglobin in micromicrograms (grams  $\times 10^{-12}$ ) present in the erythrocytes. Mean corpuscular hemoglobin concentration expresses the amount of hemoglobin in proportion to the volume of the erythrocyte and is stated in percentage.

The mean corpuscular volume obtained in this study, 96.5 cubic micra, is higher than that found in any of the other series compared in Table IV. The normal range for the age group, sex, and locality of this study is found between 82 and 107 cubic micra, since 90 per cent of the observations occurred within those limits.

These figures tend to confirm the relationship suggested by the work of Emmons<sup>6</sup> on various mammalian as well as human bloods, that the volume of the individual erythrocyte appears to vary inversely with the total number found, a larger corpuscular volume tending to compensate for the smaller number of cells.

Haden's average for mean corpuscular volume in men is 92 cubic micra. Wintrobe found 79.8 cubic micra for an average, while the mean figure calculated from Osgood's data is 86.1 cubic micra. Foster and Johnson's figure is 85.9.

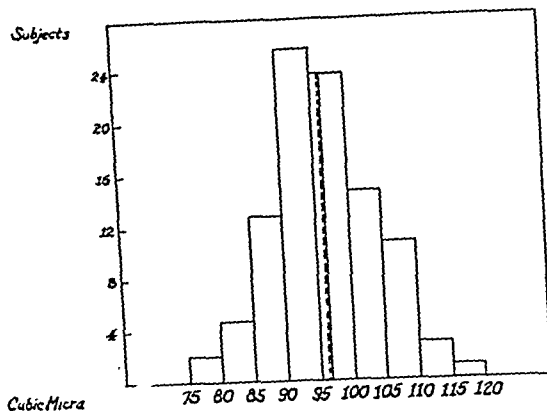


Fig. 4.—Frequency distribution of mean corpuscular volume in 100 young men. The median, 96.3, is indicated by a broken line and the mean, 96.5, by a solid line.

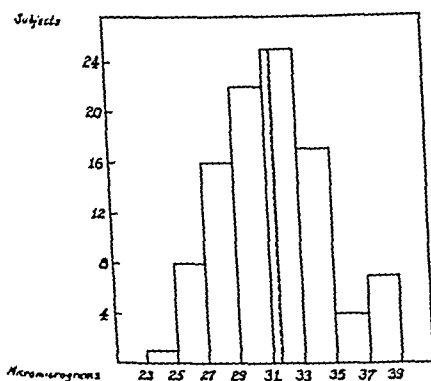


Fig. 5.

Fig. 5.—Frequency distribution of mean corpuscular hemoglobin in 100 young men. The median and the mean are identical, 31.4.

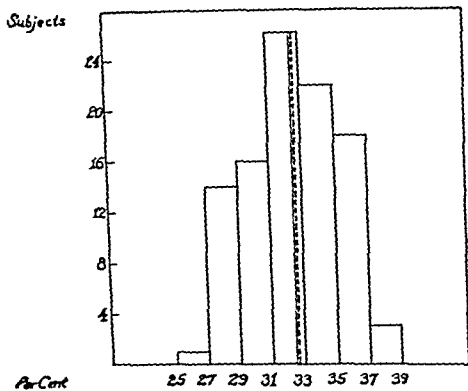


Fig. 6.

Fig. 6.—Frequency distribution of mean corpuscular hemoglobin concentration in 100 young men. The median, 32.6, is indicated by a broken line and the mean, 32.4, by a solid line.

Since the calculation of corpuscular volume, hemoglobin and hemoglobin concentration is simple and does not involve any arbitrary standard, these constants have been recommended frequently in preference to the volume, color, and saturation indices for clinical work. However, it is clear that even for the application of these terms to specific cases, well-established normals for the given conditions, age group, and sex should be known. For example, although Wintrobe<sup>36</sup> states that "values above 95 cubic micra or below 75 cubic micra are probably a manifestation of abnormality," more than half of the

normal subjects observed in the present investigation showed a corpuscular volume greater than 95 cubic micra.

The average figure for mean corpuscular hemoglobin found in the subjects of this study was 31.4 micromicrograms, with 92 per cent lying between 25 and 37 micromicrograms. This mean is almost identical with Haden's average of 31.0 micromicrograms for 35 men between eighteen and thirty, and agrees closely with the average figure found by Foster and Johnson, 30.03 micromicrograms. The same mean was observed both by Osgood and by Wintrobe and Miller, 29.2 micromicrograms.

The average found for corpuscular hemoglobin concentration was 32.4 per cent. Ninety-one per cent of the calculations lay between 27.5 and 36.5 per cent. Haden's average of 33.7 per cent and Osgood's, 33.9 per cent, are in close agreement with this figure. Wintrobe found an average of 36.6 per cent, while Foster and Johnson recorded a mean of 34.77. Horneffer calcu-

TABLE II  
MEAN ERYTHROCYTE COUNT FOR NORMAL MEN IN VARIOUS GEOGRAPHIC LOCALITIES

| INVESTIGATOR             | DATE      | NO. OF<br>SUBJECTS | LOCALITY       | MEAN<br>COUNT | AGE<br>RANGE |
|--------------------------|-----------|--------------------|----------------|---------------|--------------|
| Chamberlain <sup>3</sup> | 1911      | 687                | Philippine Is. | 5.20          | ----         |
| Emerson                  | 1921      | 176                | Baltimore      | 5.00          | 20-25        |
| Haden                    | 1922 ff.  | 70                 | Kansas City    | 4.95          | 18-50        |
| Haden                    | 1924-1930 | 230                | Kansas City    | 5.00*         | ----         |
| Komocki                  | 1924      | 17                 | Warsaw         | 5.97          | 20-40        |
| Sackett                  | 1925      | 14                 | Kansas City    | 5.09          | ----         |
| Osgood                   | 1926      | 137                | Oregon         | 5.39          | 19-30        |
| Horneffer                | 1928      | 40                 | Germany        | 4.97          | 19-29        |
| Wintrobe and Miller      | 1929      | 100                | New Orleans    | 5.85          | 20-30        |
| Parodi                   | 1930      | 50                 | Buenos Aires   | 5.50          | 18-30        |
| Cabigting                | 1930      | 22                 | Philippine Is. | 4.75          | 15 "up"      |
| Foster and Johnson       | 1931      | 115                | New Orleans    | 5.26          | 18-30        |
| Tenconi                  | 1931      | 50                 | Buenos Aires   | 5.30          | ----         |
| Price-Jones              | 1931      | 100                | London         | 5.43          | ----         |
| Chia Yu Tien†            | 1931      | 320                | Manchuria      | 5.12          | 19-64        |
| This Study               | 1933      | 100                | Kansas         | 4.84          | 20-30        |

Mean Erythrocyte Count for 2228 Men 5.189

\*These counts were made by medical students.

†Quotes Kitashima as having found 5.24 million and Sakai, 5.46 million as mean values in studies on normal Japanese.

TABLE IV  
COMPARISON OF AVERAGE NORMAL ERYTHROCYTE, HEMOGLOBIN AND PACKED CELL VOLUME  
VALUES FOUND BY VARIOUS INVESTIGATORS

| INVESTIGATOR           | LOCALITY    | NUMBER<br>SUBJECTS | AGE<br>RANGE | ERYTHRO-<br>CYTES | TOTAL<br>HB. | HB.<br>COEFF. | TOTAL<br>VOLUME | VOLUME<br>COEFF. | CORP.<br>VOL. | CORP.<br>HB. | CORP.<br>HB. CONC. |
|------------------------|-------------|--------------------|--------------|-------------------|--------------|---------------|-----------------|------------------|---------------|--------------|--------------------|
| Haden                  | Kansas      | 35                 | 18-30        | 5.05              | 15.63        | 15.48         | 46.2            | 46.0             | 92.0          | 31.0         | 33.7               |
| Osgood                 | Oregon      | 137                | 19-30        | 5.39              | 15.76        | 14.66         | 46.4*           | 43.0             | 86.1          | 29.2         | 33.1               |
| Wintrobe and<br>Miller | New Orleans | 100                | 20-30        | 5.85              | 17.0         | 14.53         | 46.5            | 39.9             | 79.8          | 29.2         | 36.6               |
| Foster and<br>Johnson  | New Orleans | 115                | 18-30        | 5.26†             | 15.63        | 14.85         | 46.7‡           | 44.4‡            | 88.8‡         | 30.0         | 34.8               |
| This study             | Kansas      | 100                | 20-30        | 4.84              | 15.12        | 15.71         | 46.5            | 48.3             | 96.5          | 31.4         | 32.4               |

\*Mean of 94 Subjects.

†Mean of 100 Subjects.

‡Heparinized Blood. Mean of 40 Subjects.



TABLE III  
MEAN HEMOGLOBIN VALUES FOR NORMAL MEN IN VARIOUS GEOGRAPHIC LOCALITIES

| MEAN HEMOGLOBIN VALUES FOR NORMAL MEN IN VARIOUS COUNTRIES      |              |         |                    |              |                                     |                              |
|-----------------------------------------------------------------|--------------|---------|--------------------|--------------|-------------------------------------|------------------------------|
| INVESTIGATOR                                                    | LOCALITY     | DATE    | NO. OF<br>SUBJECTS | AGE<br>RANGE | HEMO-<br>GLOBIN<br>GMC./100<br>G.C. | HEMO-<br>GLOBIN<br>COEFF.    |
| Harrop                                                          | Baltimore    | 1919    | 15                 | ----         | 15.05                               | ----                         |
| Haden                                                           | Kansas City  | 1922 W. | 70                 | 18-50        | 15.34                               | ----                         |
| Osgood                                                          | Oregon       | 1926    | 137                | 19-30        | 15.76                               | 13.66                        |
|                                                                 |              |         |                    |              |                                     | Osgood-Haskins;<br>Van Slyke |
| Wintrobe and Miller                                             | New Orleans  | 1929    | 100                | 20-30        | 17.0                                | 14.53                        |
| Ortiz                                                           | Argentina    | 1930    | 1104               | ----         | 14.46                               | ----                         |
| Ortiz                                                           | Argentina    | 1930    | 65                 | ----         | 15.06                               | ----                         |
| Ortiz                                                           | Argentina    | 1930    | 82                 | ----         | 15.3                                | ----                         |
| Parodi                                                          | Buenos Aires | 1930    | 50                 | 18-30        | 15.4                                | 14.0                         |
| Sokhey                                                          | Bombay       | 1930    | 121                | ----         | 15.3                                | ----                         |
| Parjono, et al.                                                 | Java         | 1930    | 19                 | ----         | 14.63                               | ----                         |
| Parjono, et al.                                                 | Java         | 1930    | 16                 | ----         | 16.12                               | ----                         |
| Foster and Johnson                                              | New Orleans  | 1931    | 115                | 18-30        | 15.63                               | 14.84                        |
| Teacott                                                         | Argentina    | 1931    | 50                 | ----         | 14.8                                | 13.9                         |
| Price-Jones                                                     | London       | 1931    | 100                | 20-51        | 14.5                                | 13.35                        |
| Dill                                                            | Boston       | 1931    | 40                 | ----         | 15.4                                | ----                         |
| Jenkins and Don                                                 | England      | 1933    | 118                | ----         | 15.85                               | ----                         |
| This Study                                                      | Kansas       | 1933    | 100                | 20-30        | 15.12                               | 15.71                        |
|                                                                 |              |         |                    |              |                                     | Van Slyke                    |
| Mean hemoglobin for 2302 men                                    |              |         |                    |              | 14.987                              |                              |
| Mean hemoglobin for 1198 men (omitting 1104 Argentine soldiers) |              |         |                    |              | 15.472                              |                              |
| Mean hemoglobin coefficient for 722 men                         |              |         |                    |              |                                     | 14.618                       |

TABLE V  
BLOOD FINDINGS IN 100 NORMAL MEN AGED 20 TO 30

| NO. | AGE | ERYTHRO-<br>CYTES | TOTAL<br>HEMOGLOBIN | HEMOGLOBIN<br>COEFF. | TOTAL CELL<br>VOLUME | VOLUME<br>COEFF. | CORPUSCULAR<br>VOLUME | CORPUSCULAR<br>HEMOGL. | CORPUSC. HEMODL.<br>CONCENTR. | COLOR<br>INDEX* | VOLUME<br>INDEX* | SATUR.<br>INDEX* |
|-----|-----|-------------------|---------------------|----------------------|----------------------|------------------|-----------------------|------------------------|-------------------------------|-----------------|------------------|------------------|
| 1   | 22  | 4.31              | 13.44               | 15.59                | 48.8                 | 56.6             | 113.2                 | 31.2                   | 27.5                          | 1.00            | 1.19             | 0.83             |
| 2   | 21  | 5.33              | 13.44               | 12.61                | 46.6                 | 43.7             | 87.4                  | 25.2                   | 28.8                          | 0.81            | 0.92             | 0.88             |
| 3   | 21  | 5.29              | 15.64               | 14.78                | 44.0                 | 41.6             | 83.1                  | 29.6                   | 35.5                          | 0.94            | 0.88             | 1.08             |
| 4   | 28  | 4.83              | 15.90               | 16.46                | 48.9                 | 50.6             | 101.2                 | 32.5                   | 32.5                          | 1.05            | 1.05             | 0.99             |
| 5   | 22  | 4.93              | 15.64               | 15.86                | 44.6                 | 45.2             | 90.4                  | 31.7                   | 35.1                          | 1.02            | 0.96             | 1.06             |
| 6   | 20  | 4.72              | 17.03               | 18.04                | 46.1                 | 48.8             | 97.7                  | 36.1                   | 36.9                          | 1.15            | 1.03             | 1.12             |
| 7   | 23  | 4.33              | 15.90               | 18.36                | 43.6                 | 50.3             | 100.7                 | 36.7                   | 36.5                          | 1.17            | 1.06             | 1.11             |
| 8   | 27  | 4.72              | 14.03               | 14.80                | 44.6                 | 47.2             | 94.5                  | 29.7                   | 31.5                          | 0.95            | 0.99             | 0.96             |
| 9   | 20  | 4.36              | 16.74               | 19.20                | 43.5                 | 49.9             | 99.8                  | 38.4                   | 38.4                          | 1.22            | 1.06             | 1.16             |
| 10  | 20  | 5.09              | 14.90               | 14.62                | 45.3                 | 44.5             | 89.0                  | 29.3                   | 32.9                          | 0.94            | 0.94             | 1.00             |
| 11  | 21  | 4.66              | 16.17               | 17.35                | 46.8                 | 50.2             | 100.4                 | 34.7                   | 34.6                          | 1.11            | 1.06             | 1.05             |
| 12  | 20  | 4.47              | 15.39               | 17.22                | 43.4                 | 48.6             | 97.1                  | 34.4                   | 35.5                          | 1.11            | 1.03             | 1.08             |
| 13  | 23  | 4.10              | 15.90               | 19.39                | 44.8                 | 54.6             | 109.3                 | 38.8                   | 35.5                          | 1.24            | 1.16             | 1.07             |
| 14  | 20  | 4.77              | 16.45               | 17.24                | 47.9                 | 50.2             | 100.4                 | 34.5                   | 34.3                          | 1.11            | 1.06             | 1.05             |
| 15  | 20  | 4.40              | 14.90               | 16.93                | 41.1                 | 46.7             | 93.4                  | 33.9                   | 36.3                          | 1.09            | 0.99             | 1.10             |
| 16  | 20  | 5.05              | 15.90               | 15.74                | 50.0                 | 49.5             | 99.0                  | 31.4                   | 31.8                          | 1.01            | 1.04             | 0.97             |
| 17  | 22  | 4.29              | 14.90               | 17.37                | 47.4                 | 55.3             | 110.5                 | 34.7                   | 31.4                          | 1.12            | 1.16             | 0.96             |
| 18  | 20  | 5.38              | 15.90               | 14.78                | 48.8                 | 45.3             | 90.7                  | 29.6                   | 32.6                          | 0.95            | 0.96             | 0.99             |
| 19  | 21  | 4.96              | 17.03               | 17.17                | 45.7                 | 46.1             | 92.1                  | 34.3                   | 27.3                          | 1.10            | 0.97             | 1.14             |
| 20  | 20  | 4.91              | 16.17               | 16.47                | 48.8                 | 49.7             | 99.4                  | 32.9                   | 33.1                          | 1.06            | 1.05             | 1.01             |
| 21  | 20  | 4.53              | 17.66               | 19.49                | 47.0                 | 51.9             | 103.8                 | 39.0                   | 37.6                          | 1.24            | 1.09             | 1.14             |
| 22  | 21  | 5.45              | 15.14               | 13.89                | 43.1                 | 39.5             | 79.1                  | 27.8                   | 35.1                          | 0.89            | 0.83             | 1.07             |
| 23  | 21  | 4.56              | 17.34               | 19.01                | 50.0                 | 54.8             | 109.6                 | 38.0                   | 34.7                          | 1.22            | 1.15             | 1.06             |
| 24  | 20  | 4.62              | 17.34               | 18.77                | 48.7                 | 52.7             | 105.4                 | 37.5                   | 35.6                          | 1.21            | 1.12             | 1.08             |
| 25  | 20  | 4.50              | 15.14               | 16.82                | 45.0                 | 50.0             | 100.0                 | 33.6                   | 33.6                          | 1.08            | 1.06             | 1.02             |
| 26  | 20  | 5.00              | 15.90               | 15.90                | 44.4                 | 44.4             | 88.8                  | 31.8                   | 35.8                          | 1.02            | 0.94             | 1.09             |
| 27  | 20  | 5.31              | 13.44               | 12.66                | 44.2                 | 41.6             | 83.2                  | 25.3                   | 30.4                          | 0.81            | 0.88             | 0.92             |
| 28  | 22  | 4.75              | 15.64               | 16.46                | 45.7                 | 48.1             | 96.2                  | 32.9                   | 34.2                          | 1.05            | 1.01             | 1.04             |
| 29  | 21  | 4.83              | 13.63               | 14.11                | 43.1                 | 44.6             | 89.2                  | 28.2                   | 28.2                          | 0.90            | 0.94             | 0.96             |
| 30  | 20  | 4.54              | 15.64               | 17.23                | 43.3                 | 47.7             | 95.4                  | 34.4                   | 36.1                          | 1.10            | 1.00             | 1.10             |
| 31  | 25  | 4.28              | 15.90               | 18.58                | 44.6                 | 52.1             | 104.2                 | 37.1                   | 35.7                          | 1.18            | 1.09             | 1.08             |
| 32  | 20  | 4.82              | 14.90               | 15.46                | 42.2                 | 43.8             | 87.6                  | 30.9                   | 35.3                          | 1.00            | 0.93             | 1.08             |
| 33  | 22  | 4.75              | 15.64               | 16.46                | 46.8                 | 49.3             | 98.5                  | 32.9                   | 33.4                          | 1.05            | 1.04             | 1.01             |
| 34  | 20  | 4.56              | 15.90               | 17.44                | 48.0                 | 52.6             | 105.3                 | 34.9                   | 33.1                          | 1.12            | 1.11             | 1.01             |
| 35  | 22  | 4.91              | 16.74               | 17.05                | 50.0                 | 50.9             | 101.8                 | 34.1                   | 33.5                          | 1.09            | 1.07             | 1.02             |
| 36  | 20  | 4.50              | 15.14               | 16.82                | 43.9                 | 48.8             | 97.6                  | 33.6                   | 34.5                          | 1.08            | 1.03             | 1.04             |
| 37  | 21  | 4.42              | 13.63               | 15.42                | 47.1                 | 53.3             | 106.6                 | 30.8                   | 28.9                          | 0.99            | 1.13             | 0.88             |
| 38  | 20  | 4.60              | 14.90               | 16.20                | 46.5                 | 50.5             | 101.1                 | 32.4                   | 32.0                          | 1.04            | 1.06             | 0.98             |
| 39  | 20  | 4.52              | 13.07               | 14.46                | 47.9                 | 53.0             | 106.0                 | 28.9                   | 27.3                          | 0.92            | 1.11             | 0.83             |
| 40  | 21  | 4.72              | 17.34               | 18.37                | 46.8                 | 49.6             | 99.2                  | 36.7                   | 37.1                          | 1.18            | 1.05             | 1.12             |
| 41  | 23  | 5.06              | 15.14               | 14.96                | 46.1                 | 45.6             | 91.1                  | 29.9                   | 32.8                          | 0.96            | 0.96             | 1.00             |
| 42  | 21  | 4.72              | 14.22               | 15.05                | 43.7                 | 46.3             | 92.6                  | 30.1                   | 32.5                          | 0.97            | 0.98             | 0.99             |
| 43  | 25  | 5.13              | 13.82               | 13.46                | 42.7                 | 41.6             | 83.2                  | 26.9                   | 32.4                          | 0.86            | 0.87             | 0.99             |
| 44  | 21  | 5.08              | 14.44               | 14.21                | 47.4                 | 46.7             | 93.3                  | 28.4                   | 30.5                          | 0.92            | 0.99             | 0.93             |
| 45  | 26  | 4.88              | 14.22               | 14.53                | 46.8                 | 48.0             | 95.9                  | 29.1                   | 30.4                          | 0.93            | 1.01             | 0.92             |
| 46  | 22  | 5.55              | 16.45               | 14.82                | 47.0                 | 42.3             | 84.7                  | 29.6                   | 35.0                          | 0.95            | 0.89             | 1.07             |
| 47  | 21  | 4.56              | 13.44               | 14.74                | 41.1                 | 45.1             | 90.1                  | 29.5                   | 32.7                          | 0.95            | 0.96             | 0.99             |
| 48  | 20  | 5.01              | 14.67               | 14.64                | 48.4                 | 48.3             | 96.6                  | 29.3                   | 30.3                          | 0.94            | 1.02             | 0.92             |
| 49  | 22  | 5.10              | 13.63               | 13.36                | 45.9                 | 45.0             | 90.0                  | 26.7                   | 29.9                          | 0.85            | 0.95             | 0.90             |
| 50  | 28  | 5.33              | 13.82               | 12.95                | 49.4                 | 46.3             | 92.7                  | 25.9                   | 28.0                          | 0.83            | 0.97             | 0.86             |
| 51  | 23  | 4.49              | 14.03               | 15.59                | 47.4                 | 52.8             | 105.6                 | 31.2                   | 29.6                          | 1.00            | 1.11             | 0.90             |
| 52  | 24  | 4.88              | 15.14               | 15.51                | 48.9                 | 50.1             | 100.2                 | 31.0                   | 31.0                          | 0.99            | 1.05             | 0.94             |
| 53  | 20  | 4.89              | 16.74               | 17.12                | 50.0                 | 51.1             | 102.2                 | 34.2                   | 33.5                          | 1.08            | 1.07             | 1.02             |
| 54  | 26  | 4.88              | 15.90               | 16.29                | 43.9                 | 45.0             | 90.0                  | 32.6                   | 36.2                          | 1.04            | 0.95             | 1.09             |

\*For the calculation of these indices, 15.59 gm. of hemoglobin and 47.4 c.c. of packed cells have been considered as 100 per cent.

TABLE V—CONT'D

| NO.  | AGE | ERYTHRO-<br>CYTES | TOTAL<br>HEMOGLOBIN | HEMOGLOBIN<br>COEFF. | TOTAL CELL<br>VOLUME | VOLUME<br>COEFF. | CORPUSCULAR<br>VOLUME | CORPUSCULAR<br>HEMOG. | CORPUSC. HEMOG.<br>CONCENTR. | COLOR<br>INDEX* | VOLUME<br>INDEX* | SATUR.<br>INDEX* |
|------|-----|-------------------|---------------------|----------------------|----------------------|------------------|-----------------------|-----------------------|------------------------------|-----------------|------------------|------------------|
| 55   | 23  | 4.99              | 17.03               | 17.06                | 49.6                 | 49.7             | 99.4                  | 34.1                  | 34.3                         | 1.09            | 1.05             | 1.04             |
| 56   | 25  | 4.54              | 15.14               | 16.68                | 46.9                 | 51.7             | 103.3                 | 33.3                  | 32.3                         | 1.07            | 1.09             | 0.98             |
| 57   | 21  | 4.27              | 13.63               | 15.96                | 46.8                 | 54.8             | 109.6                 | 31.9                  | 29.1                         | 1.02            | 1.16             | 0.88             |
| 58   | 21  | 4.88              | 13.25               | 13.58                | 43.5                 | 44.6             | 89.1                  | 27.2                  | 30.5                         | 0.87            | 0.94             | 0.92             |
| 59   | 21  | 4.97              | 15.64               | 15.73                | 45.9                 | 46.2             | 92.4                  | 31.7                  | 34.1                         | 1.01            | 0.98             | 1.03             |
| 60   | 23  | 4.80              | 15.39               | 16.03                | 44.7                 | 46.6             | 93.1                  | 32.1                  | 34.4                         | 1.03            | 0.98             | 1.05             |
| 61   | 20  | 4.74              | 13.63               | 14.38                | 43.5                 | 45.9             | 91.8                  | 28.8                  | 31.3                         | 0.92            | 0.97             | 0.95             |
| 62   | 20  | 4.67              | 13.44               | 14.39                | 45.6                 | 48.8             | 97.6                  | 28.8                  | 29.5                         | 0.92            | 1.03             | 0.90             |
| 63   | 20  | 5.03              | 14.03               | 13.95                | 47.5                 | 47.2             | 94.4                  | 27.9                  | 29.5                         | 0.89            | 0.99             | 0.90             |
| 64   | 20  | 4.89              | 14.44               | 14.77                | 48.9                 | 50.0             | 100.0                 | 29.5                  | 29.5                         | 0.95            | 1.05             | 0.90             |
| 65   | 21  | 4.53              | 14.67               | 16.15                | 45.8                 | 50.6             | 101.2                 | 32.4                  | 32.0                         | 1.03            | 1.07             | 0.97             |
| 66   | 22  | 4.99              | 13.44               | 13.47                | 47.8                 | 47.9             | 95.8                  | 26.9                  | 28.1                         | 0.86            | 1.01             | 0.85             |
| 67   | 22  | 4.85              | 13.82               | 14.24                | 47.9                 | 49.4             | 98.8                  | 28.5                  | 28.9                         | 0.92            | 1.04             | 0.88             |
| 68   | 22  | 5.45              | 12.89               | 11.83                | 51.1                 | 46.9             | 93.8                  | 23.7                  | 25.2                         | 0.76            | 0.99             | 0.77             |
| 69   | 20  | 5.14              | 13.07               | 12.71                | 48.0                 | 46.7             | 93.4                  | 25.4                  | 27.2                         | 0.82            | 0.98             | 0.83             |
| 70   | 20  | 5.23              | 16.17               | 15.46                | 44.7                 | 42.7             | 85.5                  | 30.9                  | 36.2                         | 0.99            | 0.90             | 1.11             |
| 71   | 23  | 4.83              | 16.74               | 17.33                | 46.5                 | 48.2             | 96.3                  | 34.7                  | 36.0                         | 1.10            | 1.01             | 1.08             |
| 72   | 20  | 4.92              | 14.03               | 14.26                | 40.7                 | 41.4             | 82.7                  | 28.5                  | 34.5                         | 0.92            | 0.88             | 1.05             |
| 73   | 30  | 4.68              | 18.00               | 19.23                | 51.6                 | 55.1             | 110.3                 | 38.5                  | 34.9                         | 1.22            | 1.15             | 1.06             |
| 74   | 22  | 4.68              | 14.90               | 15.92                | 46.0                 | 49.2             | 98.3                  | 31.8                  | 32.4                         | 1.02            | 1.04             | 0.99             |
| 75   | 21  | 5.08              | 18.70               | 18.41                | 51.0                 | 50.2             | 100.4                 | 36.8                  | 36.7                         | 1.18            | 1.06             | 1.11             |
| 76   | 21  | 4.87              | 15.39               | 15.80                | 45.1                 | 46.3             | 92.6                  | 31.6                  | 34.1                         | 1.02            | 0.98             | 1.04             |
| 77   | 20  | 5.17              | 14.56               | 14.08                | 48.8                 | 47.2             | 94.4                  | 28.2                  | 29.8                         | 0.90            | 1.00             | 0.90             |
| 78   | 22  | 4.86              | 15.02               | 15.45                | 45.0                 | 46.3             | 92.6                  | 30.9                  | 33.4                         | 0.99            | 0.98             | 1.01             |
| 79   | 20  | 5.15              | 15.14               | 14.70                | 48.5                 | 47.1             | 94.2                  | 29.4                  | 31.2                         | 0.94            | 0.99             | 0.95             |
| 80   | 21  | 4.61              | 14.13               | 15.33                | 44.4                 | 48.9             | 96.3                  | 30.7                  | 31.8                         | 0.99            | 0.98             | 0.97             |
| 81   | 20  | 4.27              | 13.63               | 15.96                | 49.3                 | 57.7             | 115.5                 | 31.9                  | 27.6                         | 1.02            | 1.22             | 0.84             |
| 82   | 21  | 4.43              | 13.92               | 15.71                | 48.7                 | 55.0             | 109.9                 | 31.4                  | 28.6                         | 1.00            | 1.15             | 0.86             |
| 83   | 21  | 4.90              | 15.90               | 16.22                | 48.8                 | 50.0             | 99.6                  | 32.4                  | 32.6                         | 1.04            | 1.05             | 0.99             |
| 84   | 25  | 4.94              | 15.39               | 15.58                | 46.9                 | 47.5             | 94.9                  | 31.2                  | 32.8                         | 1.00            | 1.00             | 1.00             |
| 85   | 22  | 4.99              | 14.44               | 14.47                | 47.4                 | 47.5             | 94.9                  | 28.9                  | 30.5                         | 0.93            | 1.00             | 0.93             |
| 86   | 26  | 5.05              | 14.44               | 14.30                | 48.0                 | 47.5             | 95.0                  | 28.6                  | 30.1                         | 0.92            | 1.00             | 0.92             |
| 87   | 22  | 5.39              | 17.34               | 16.09                | 53.8                 | 49.9             | 99.8                  | 32.2                  | 32.2                         | 1.03            | 1.06             | 0.97             |
| 88   | 26  | 4.32              | 14.90               | 17.25                | 45.7                 | 52.9             | 105.8                 | 34.5                  | 32.6                         | 1.12            | 1.13             | 0.99             |
| 89   | 23  | 5.19              | 13.72               | 13.22                | 49.0                 | 47.2             | 94.4                  | 26.4                  | 28.0                         | 0.85            | 0.99             | 0.85             |
| 90   | 26  | 5.05              | 14.03               | 13.89                | 44.4                 | 44.0             | 87.9                  | 27.8                  | 31.6                         | 0.89            | 0.93             | 0.96             |
| 91   | 24  | 4.78              | 14.67               | 15.35                | 44.7                 | 46.8             | 93.5                  | 30.7                  | 32.8                         | 0.98            | 0.98             | 1.00             |
| 92   | 28  | 4.95              | 14.90               | 15.05                | 43.5                 | 44.0             | 87.9                  | 30.0                  | 34.3                         | 0.97            | 0.93             | 1.04             |
| 93   | 29  | 4.85              | 13.82               | 14.25                | 49.0                 | 50.5             | 101.0                 | 28.5                  | 28.2                         | 0.92            | 1.06             | 0.86             |
| 94   | 22  | 4.86              | 14.56               | 14.98                | 45.0                 | 46.4             | 92.6                  | 30.0                  | 32.4                         | 0.96            | 0.98             | 0.98             |
| 95   | 26  | 4.58              | 15.02               | 16.40                | 47.4                 | 51.7             | 103.5                 | 32.8                  | 31.7                         | 1.04            | 1.09             | 0.96             |
| 96   | 21  | 5.11              | 16.02               | 15.68                | 48.2                 | 47.2             | 94.3                  | 31.4                  | 33.2                         | 1.01            | 1.00             | 1.01             |
| 97   | 21  | 5.35              | 16.59               | 15.51                | 47.9                 | 44.8             | 89.5                  | 31.0                  | 34.6                         | 0.99            | 0.94             | 1.05             |
| 98   | 27  | 4.61              | 14.67               | 15.91                | 44.4                 | 48.2             | 96.3                  | 31.8                  | 33.0                         | 1.02            | 1.02             | 1.00             |
| 99   | 23  | 5.23              | 14.56               | 13.91                | 49.5                 | 47.3             | 94.6                  | 27.8                  | 29.4                         | 0.89            | 0.99             | 0.89             |
| 100  | 25  | 5.19              | 16.31               | 15.71                | 46.5                 | 44.8             | 89.6                  | 31.4                  | 35.1                         | 1.01            | 0.94             | 1.07             |
| Mean |     | 4.84              | 15.12               | 15.71                | 46.5                 | 48.3             | 96.5                  | 31.4                  | 32.4                         | 1.01            | 1.02             | 0.99             |

lated a mean corpuscular hemoglobin of 32.4 for his series of 40 healthy men. The criticism of his method of hemoglobin determination has already been mentioned.

#### THE QUESTION OF GEOGRAPHIC VARIATIONS

The comparatively wide differences which have been found to exist in hemoglobin and erythrocyte count have frequently raised the question of possible geographic variations other than the well-recognized increase with altitude.

Parjono, Radsma and Joenoes<sup>25</sup> compiled data on blood iron and oxygen capacity in an effort to find some correlation between the amount of hemoglobin and geographic latitude. The data used were found insufficient to establish any clear relationship.

In Tables II and III are listed erythrocyte and hemoglobin determinations from various geographic localities. From these figures it is apparent that any correlation on the basis of geographic locality is very difficult, since in several cases, investigators working in the same locality have secured widely differing results. There are, however, few striking differences.

Many other factors than geographic location are doubtless existent as, for example, in the case of the difference found between poorer natives and the better-situated students and native doctors in the Java group of Parjono, et al. For this particular difference these authors suggest the prevalence of intestinal worms among the poorer classes as a possible explanation.

Orias<sup>22</sup> found a definite difference between common soldiers, officers and students in Buenos Aires (Table III). He concludes that social condition is an important factor in hemoglobin level. Horneffer also noted this difference, finding 16.25 gm. as the mean value for 20 students and 15.80 gm. as the mean for 20 soldiers.

Price-Jones suggests chronic carbon monoxide poisoning from motor cars, with an accompanying compensatory increase in hemoglobin and red blood cells to account for the difference in hemoglobin content which was found by him in London and Boston. This explanation is claimed by Jenkins and Don to have been invalidated by observations made on mechanics. These investigators find an even higher mean hemoglobin value for individuals from various localities in England than Price-Jones found in Americans.

The possibility of a difference in hemoglobin level between smokers and nonsmokers due to carbon monoxide has also been investigated,<sup>32a</sup> but no significant difference was found.

Some significant differences due to technic would seem inevitable in any list of investigations as large as those quoted in Tables II and III. Further sampling of the normal population on an extensive scale is needed to clarify the question of normal standards for general use.

#### SUMMARY

1. Careful observations made on 100 healthy men between the ages of twenty and thirty in Kansas show a mean erythrocyte count of 4.84 million, 15.12 gm. of hemoglobin per 100 c.c. of blood and 46.5 c.c. of packed cells per 100 c.c. of blood.

2. When a hemoglobin coefficient of 15.59 gm. and a volume coefficient of 47.4 c.c. are employed as 100 per cent standards, the color, volume and saturation indices are approximately 1.

3. Calculations from the above data give the following average figures: for mean corpuscular volume, 96.5 cubic micra; for mean corpuscular hemoglobin, 31.4 micromicrograms; for mean corpuscular hemoglobin concentration, 32.4 per cent.

4. Comparison of erythrocyte and hemoglobin values found by investigators in widely separated areas shows no apparent relation to geographic location. Technical variations may be one of the important factors in observed differences.

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## WUCHERERIA (FILARIA) BANCROFTI INFECTION IN MAN WITH AN UNUSUAL HISTORY\*

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### CASE REPORT

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THE occurrence of Wuchereria infection in a person who has remained within the vicinity of the District of Columbia all of his life is so unusual that we feel justified in reporting it.

We have not been able to discover all the links in the chain of circumstances leading to the infection. This, together with the usually accepted epidemiology of filariasis, while incidentally serving to remind the general practitioner of its possible occurrence in this part of the country, also serves to focus more interest of the entomologist on the way or ways in which the patient may have become infected. From an epidemiologic standpoint we thought of four possibilities: (1) a wider general distribution of species of mosquitoes generally known to serve as vector for this disease; (2) a special means of distribution by these same mosquitoes laying their eggs in the few centimeters of water frequently found in the bottom of the lifeboats on freighters coming from endemic ports with a crew, some of which may be carriers; the larvae from these eggs become mosquitoes in time to bite the crew and they, after the usual incubation period, infect persons in any subsequent port; (3) the common mosquito of this vicinity, which may assume the rôle of vector when in contact with carriers from an endemic area; (4)

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and lastly, some species of tick, which may serve as vectors. The last possibility is of interest chiefly because *Filaria perstans*, an organism very much like *Wuchereria bancrofti*, except that it does not have a sheath, is transmitted by a certain species of tick, and recently we have had an increase in tick (Eastern type of Rocky Mountain spotted) fever in this vicinity.

#### CASE REPORT

X. Y., a negro janitor, aged twenty-nine years, was admitted to Freedman's Hospital, May 15, 1933, with a chief complaint of progressive enlargement of the scrotum and right leg and foot with several areas of suppuration from the scrotal sac, one on the right buttock, and glandular enlargement and ulceration of the left axillary region.

The past history is important in that the patient has lived in Washington all of his life and has never travelled more than one hundred miles from Washington, and when he did leave the city, it was only for a short time, generally on fishing trips.

He has had measles, mumps, whooping cough, and eczema during childhood, but has otherwise enjoyed good health up to the beginning of the present illness. His appetite has remained good. He has used alcohol and tobacco in moderation, but no narcotics.

The only contacts which he can recall with the tropics or subtropical zones are: prior to the present illness he had worked as janitor for a family of Filipinos, then living in Washington; and his family at various times received packages, some of which contained plants, from relatives in Mississippi. The family history is essentially negative except for an almost complete progressive blindness in his father (cataract).

*Present Illness.*—About five years ago the patient, without any previous chill, fever or other symptoms of illness, began to have slight pains in the umbilical region. This continued for some time. Shortly after this he observed that his scrotum was enlarging. This continued, and about two weeks after the enlargement was noticed, two small areas of ulceration were observed where the scrotal walls rubbed against the thighs.

At this time his wife left him, accusing him of having some type of venereal disease.

He was treated by a private physician for about one year with various local and internal medications, but when the scrotum continued to enlarge, he was hospitalized for ten weeks at Gallinger Municipal Hospital. There, various studies and treatments were performed without any check of the progressive enlargement of the scrotum. They did not find microfilariae in the blood. His general condition of health was good up to the time of admission to Gallinger Hospital.

After leaving the hospital, he did not return to work because the size of the scrotum and the several areas of ulceration made walking more difficult.

For the last four years he has not worked nor received any systematic medical treatment. During this time the scrotum has continued to enlarge. Areas of suppuration have developed over the scrotum and areas of excoriation over the inner surface of the thighs where they rub against the scrotal wall. Glandular enlargement developed in the axillae with subsequent suppuration of the left axilla. About this time a small ulceration developed over the right buttock and about two years ago the right leg and foot began to enlarge and to become progressively rough.

In the light of the above progress he was advised by a member of the staff of Freedman's Hospital to come into the hospital for study. This he decided to do and was admitted May 15, 1933.

*Physical Examination.*—The general examination revealed a very obese negro male weighing more than 350 pounds, without any evidence of marked discomfort or illness, who was in a jovial mood. The head and neck were normal. The chest and upper extremities, except for some glandular enlargement in each axilla and slight suppuration of the left axilla, were normal. The abdomen showed abundant adipose tissue.

Genital examination revealed a very greatly enlarged scrotum, appearing to weigh about 25 pounds. It was deeply pigmented here and there, nodular and hard in places. Other

areas reflected light due to the drying of serum which had oozed from its surface, while still others presented deep sinuses covered by a purulent exudate. Where the folds of pendulous abdominal fat met the fat on the anterior surface of the thigh on either side, the valleys of the inguinal regions presented ulcerated surfaces penetrated by tunneled sinuses. These showed a marked tendency to granulation. While the penis was completely invaginated within the overgrowth of scrotal tissue, there seemed to be a marked proliferation of prepubic tissue, which was unusually pendulous. There were areas of excoriation over the inner surface of each thigh, where the very much enlarged scrotum rubbed against them. The right buttock showed a small area of suppuration. The left leg and foot, except for the marked dryness and scales, were apparently normal. The right leg was larger around by two inches at the calf and knee than the normal leg. The right leg and foot showed scaling and pigmen-



Fig. 1.—A, Before treatment. Measurements: Left, thigh 32 inches; knee 22 inches; calf 20 $\frac{1}{4}$  inches; ankle 14 $\frac{1}{2}$  inches. Right, thigh 32 $\frac{1}{4}$  inches; knee 24 inches; calf 22 inches; ankle 14 inches. B, After treatment.

tion together with a few shining areas in which serum appeared to be oozing. There was no definite ulceration of either limb.

A clinical diagnosis of elephantiasis was made by the staff.

*Laboratory Examination.*—Several thick smears of blood taken during the night, one at 9:15 P.M. and another at 10:30 P.M. on separate nights, showed microfilaria when stained by the Giemsa method. The coagulation time was five minutes. Red blood corpuscles 4,670,000; white blood cells 14,000. Differential: Small lymphocytes 36 per cent, large lymphocytes 25 per cent, large mononuclears 8 per cent, transitionals 6 per cent, polymorphonuclear neutrophils 20 per cent, eosinophils 5 per cent. Blood Wassermann was negative. Urine showed no microfilariae, a few epithelial cells, and a positive indican test; otherwise nothing significant.



Stools were free from parasites, ova or larvae. Bacteriologic culture from several of the areas of ulceration and from some of the deep tissue removed at operation showed various micrococci, *Staphylococcus albus*, a nonhemolytic streptococcus, which fermented mannite and was considered to be *Streptococcus fecalis*; from one area there was a diphtheroid organism. The condition of the patient while in the hospital was uneventful. The earlier treatment consisted primarily of Sitz bath and mild local antiseptics in an attempt to clear up most of the secondary infection.

Attempts were made on two occasions to drain fluid from the enlarged serotum. Several punctures with various sized needles failed to locate any definite pockets of fluid. We interpreted the enlarged serotum to be due to chronic edema, and proliferation of the subcutaneous connective tissue secondary to this edema, the pathologic basis for which is the blocking of the lymph circulation.

The patient was sufficiently clear of secondary infection by June 2, 1933, to warrant an operation. On June 2, 1933, under general ether anesthesia, the enlarged serotum was removed together with some of the inguinal lymphatics. Also an extensive section of the prepubic redundancy was removed, effecting complete recovery of the penis. It was found that the skin which covered the penis was not thickened, but only evaginated, due to the weight of the involved serotum. The testicles were found to be normal in every respect. They were stripped

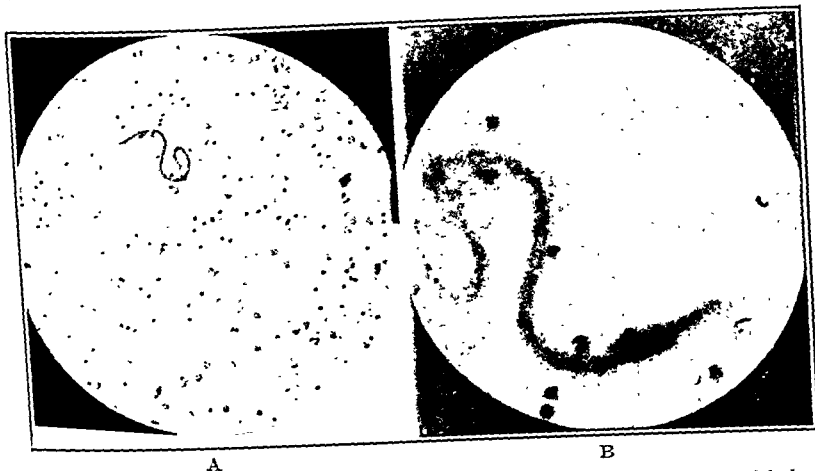


Fig. 2.—A. Shows a low-power magnification of the *Microfilaria* stained with hematoxylin as described above. B. Shows the same *Microfilaria* under a higher magnification.  
The Technic of Preparation Was as Follows.—Slides were made from the centrifuged specimens of citrated blood, which had also been dehemoglobinized by distilled water. The residue was used for the smear. The smear was fixed in a solution containing equal parts of alcohol and ether for five minutes; stained with warm hematoxylin for eight minutes, decolorized for two seconds in 80 per cent alcohol to which had been added a few drops of hydrochloric acid and dried for examination.

of all of the proliferative tissue and covered in a new sac by joining the healthy skin of the perineum and inner side of the thigh to the normal skin covering the penis. For several weeks the penis was visible. Erection was noted on many occasions. Failure to remove sufficient subcutaneous tissue in the prepubic region encouraged the return of redundancy with resulting invagination of the penis. The patient had an uneventful convalescence from the operation. He was discharged from the hospital July 7, 1933, markedly improved, with instructions to return at intervals to the out-patient department for observation and further treatment.

#### DISCUSSION

In view of the nodular swellings under the arms and elsewhere, we were compelled to consider a probable onchocerciasis, a rare disease due to *Onchocerca volvulus*, a member of filariidae family, and transmitted by some gnat of the Simuliidae family.

The geographic distribution of the onchocerciasis, as well as the history of this case, made that solution highly improbable from the beginning, and the microscopic study of the microfilaria definitely ruled it out.

Clinically the case was one of elephantiasis. We therefore had to consider the etiology from three standpoints: infection with *Wuchereria bancrofti* and a secondary invader which some believe to be primarily responsible for the ulceration and much of the lymphatic blockage or elephantiasis of a nonparasitic type resembling the cases reported by Gager,<sup>1</sup> and others. While the works of O'Connor and Hulse,<sup>2</sup> and O'Connor<sup>3</sup> seem to show that elephantiasis in *Wuchereria* infection may occur without secondary infection, we believe that this case is one in which the secondary infection by the streptococci and staphylococci is an important secondary etiologic factor in producing the syndrome.

The question of where and by what means the patient received the infection seems to be the most important consideration here.

*Wuchereria bancrofti* infection, while common in various areas throughout the tropics and subtropical belts, is most important as a United States menace because of its prevalence in the Islands and ports of the Caribbeans and its endemicity in Charleston, South Carolina. Man is the only proved intermediate host harboring the adult worms and the sexual phases, and the mosquito is the only proved definitive host in which complete development may take place, and is thus the vector of interest.

Since we have evidence that man cannot infect man directly, but that the microfilariae must undergo a certain period and complete certain stages of development in the mosquito before the infective form is produced and is thus ready to be transmitted to another person, the vector becomes the important problem here. There are at least four known genera of the order Diptera in which the complete development of the *Wuchereria bancrofti* is known to be possible: viz., *Aedes*, *Anopheles*, *Culex*, and *Mansonia*. There are several species under each of these genera in which the complete development of *Wuchereria* is known to be possible, but for all practical purposes in the United States only three species are important as vectors. They are *Culex fatigans*, common in the West Indies and South Carolina; *Anopheles (Nyssorhynchus) albimanus*, common in the Caribbean; and *Aedes variegatus*, common in the Pacific Islands. While such species as *Anopheles costalis* are very efficient vectors, a geographic consideration of its habitat, primarily confined to the West Coast of Africa, the small amount of travel and trade between those ports and North America and the long distance make it a vector of almost negligible consideration in this case. For many of the same reasons, *Aedes variegatus* becomes less likely as a vector in this case. Using the above methods of reasoning, we feel that the frequent contact between Baltimore and the Caribbean and Charleston, South Carolina, makes the *Culex fatigans* or *Anopheles albimanus* the most likely vector in this case.

The question of this man as a carrier and a menace is worthy of mention only to rule out the danger. As pointed out by Manson-Bahr,<sup>4</sup> when fewer than one microfilaria were present in 2 c.c. of the patient's blood, the appro-

priate mosquito frequently failed to acquire infection. In this case, there was about one microfilaria per 3 c.c. of blood as averaged by our concentration method before operation and about one microfilaria per 5 c.c. of blood now. The usually accepted vectors are infrequent in this locality. We also know that some of the microfilariae are destroyed during their stay in the mosquito.

These facts together with the fact that both a male and a female must become lodged in the same area in man and then grow and develop their reproductive organs preparatory to bringing about a fecundated adult female parent before multiplication can take place, make this case insignificant as a danger. However, if the patient were more heavily infected, he would be a potentially dangerous carrier.

There is no accepted medication. Many clinics in the tropics report the trial and failure of various intravenous medications. I was impressed with the reports of success still claimed by the staff of the Municipal Hospital of Fredericksted, St. Croix, Virgin Islands, while visiting there during the summer of 1932. The other hospitals in various parts of the Caribbean did not report such results. While the surgical treatment selected in this case is primarily palliative and of no unusual significance, untold individual aid has been rendered this patient.

#### SUMMARY AND CONCLUSION

*Wuchereria bancrofti* infection may occur in persons living in the temperate zones who have not had any obvious contact with the commonly accepted vector. Such a case is here reported.

We wish to express our appreciation to Doctors William H. Taliaferro and Clay G. Huff of the Department of Parasitology, University of Chicago, for their opinion in regard to this species of *Microfilaria*, and to Dr. Porter B. Lennox of the Department of Pathology, School of Medicine, Howard University, for taking and developing the photomicrographs.

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## THE TOXIN OF BACILLUS PROTEUS\*

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THE toxic properties of cultures of proteus isolated from various sources has been the subject of considerable study since the time of Hauser's<sup>1</sup> original description of the type organism of the proteus group when he demonstrated its pathogenicity for animals.

That both solutions of the dried, sterile, powdered organisms and filtrates from cultures have definitely toxic effects has been repeatedly demonstrated, and Bengston<sup>2</sup> in summarizing the results of the work in this field states that "*B. proteus vulgaris* produces a weak soluble toxin, as compared with tetanus and diphtheria."

However, while agglutinins have been demonstrated in high dilutions following immunization and in infections with the organism, and while a relative immunity to the usual fatal doses has been produced, there seems to have been only one demonstration of an antitoxin. Herter and Ten Broeck<sup>3</sup> were unable to establish immunity to the toxic extracts of the powdered organisms.

Isabolinski and Judenitsch<sup>4</sup> succeeded in preparing two culture filtrates containing immunizing antibodies of different degrees of effectiveness. The cultures were obtained from patients suffering from typhus fever and were grown on agar plates and in broth. The "H" form contained more immunizing substances than the "O" form, as shown on rabbits receiving lethal doses of the original culture.

I have investigated the subject of antitoxin production in rabbits and attempted to form some idea of the relative toxicity of the filtrates for man.

The strain of proteus employed was isolated from catheterized bladder and ureteral urine from a girl, twenty years old, with uncomplicated pyelitis due to proteus.

The organism was a small gram-negative rod, motile in young cultures, gradually losing motility after the first twenty-four hours. On agar it spread rapidly over the plates forming a thin gray film, covering the plate from four to five streaks in twenty-four hours. It grew more rapidly on blood agar than plain. It liquefied gelatin, naptiform in twenty-four hours, and complete in from three to four days at room temperature. Litmus milk was changed to acid and coagulated in from two to three days, peptonized and alkaline in five days. Nitrites were formed. No indol was produced. Acid and gas are formed in dextrose, galactose, levulose and saccharose (after three days). No acid or gas was produced on lactose, maltose, raffinose, arabinose, mannitol, dulcitol, dextrin, glycerol, salicin, sorbitol and inulin. Sugar mediums were prepared by adding 5 per cent sugar to broth containing 1 per cent Liebig's meat extract 5 per cent Witte's peptone, and sodium chloride 5 per cent.

\*From the John McCormick Institute for Infectious Diseases, Chicago.  
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Plain broth mediums, as used in fermentation tests, titrated to  $P_H$  6.0 were used for growing cultures in preparation of the filtrates. Cultures were incubated from one to eleven days at  $37^\circ C$ . Each culture was checked for contamination before filtering. Berkefeld "X" filters were used for the first filtration; the filtrate was then passed through "W" filters and tested for sterility by both aerobic and anaerobic methods, culturing on blood agar slants.

#### EFFECT UPON RABBITS

The rabbits used were young and weighed between 1,500 and 2,000 gm., averaging about 1,700 gm. The filtrate was injected, unless otherwise specified, into the marginal ear vein.

Ten cubic centimeters of the filtrate of a one-day culture caused no apparent ill effect. Ten cubic centimeters of the filtrate of a three-day culture caused death in less than twenty-four hours. The same dose of the filtrate of a five-day culture failed to kill another rabbit but produced severe illness with diarrhea and moderate loss of weight. Five cubic centimeters of the filtrate of an eight-day culture caused death in less than twenty-four hours and in 6 rabbits all doses above this were fatal in less than twenty-four hours. Doses

TABLE I

THE EFFECT UPON RABBITS OF FILTRATES OF CULTURE GROWN FOR VARYING LENGTHS OF TIME WITH VARIATIONS IN THE DOSAGE. INTRAVENOUS ADMINISTRATION

| RABBIT | DOSE IN C.C. | AGE OF CULTURE         | RESULT                                                                                                                                             |
|--------|--------------|------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|
| 1      | 11           | Broth control          | No ill effects.                                                                                                                                    |
| 2      | 10           | 1 day                  | No ill effects.                                                                                                                                    |
| 3      | 10           | 3 days                 | Died in less than 18 hours. Heart's blood sterile. This rabbit had received 10 c.c. of broth control two days before without apparent ill effects. |
| 4      | 10           | 5 days                 | Diarrhea for four days, loss of 250 gm. Recovery.                                                                                                  |
| 5      | 5            | 8 days                 | Died in less than 18 hours.                                                                                                                        |
| 6      | 9            | 11 days                | Very ill next day. Emaciation with loss of considerable weight. Died in 16 days.                                                                   |
| 7      | 5            | 11 days                | Died in less than 18 hours.                                                                                                                        |
| 8      | 10           | 11 days                | Died in less than 18 hours.                                                                                                                        |
| 9      | 10           | 11 days                | Died in less than 18 hours.                                                                                                                        |
| 10     | 10           | 11 days                | Died in less than 18 hours.                                                                                                                        |
| 11     | 10           | Broth control          | No ill effects.                                                                                                                                    |
| 12     | 10           | Broth control          | No ill effects.                                                                                                                                    |
| 13     | 1½           | 11 days                | Died in two days; 13 c.c. of blood had been withdrawn 2 days before without apparent ill effects.                                                  |
| 14     | 1            | 11 days                | Died in 24 hours; 13 c.c. of blood had been withdrawn 2 days before without apparent ill effects.                                                  |
| 15     | 2            | 11 days<br>(3 mo. old) | Lost 330 gm. in 4 days. Diarrhea was present on the first day and more marked on the second. Lost 610 gm. in 7 days.                               |
| 16     | 2            | 11 days<br>(3 mo. old) | Found dead in less than 15 hours.                                                                                                                  |

of  $\frac{3}{4}$  to 2 c.c. of a three-month-old filtrate killed 3 rabbits and produced severe illness in another. Doses of  $\frac{3}{4}$  c.c. and  $1\frac{1}{2}$  c.c. killed 2 rabbits from which 10 c.c. of heart blood had been withdrawn two and nine days before, respectively.

One rabbit recovered from intramuscular injection of 5 c.c. of toxin which, when injected intravenously, killed rabbits of the same weight when administered in doses of less than 2 c.c. The rabbit injected intramuscularly, however, was sick and lost 20 per cent of its weight.

To determine the toxin production in gelatin, cultures were grown for six weeks at room temperature, diluted with salt solution in proportion 1:4 and filtered as described for broth filtrates;  $1\frac{1}{2}$  c.c. of this dilution killed a rabbit in less than twenty-four hours. One cubic centimeter caused profound illness, with loss of 29 per cent of weight in three days. Control gelatin produced no ill effects.

The results (Table I) demonstrate that less than 5 c.c. of filtrate from the eleven-day broth is usually a fatal dose for a rabbit weighing between 1,500 and 2,000 gm. Doses of 2 c.c. or less caused either death or profound illness. Doses of 10 c.c. always killed in less than twenty-four hours.

#### TOXIC EFFECTS

The most characteristic reaction to the filtrate was diarrhea, the entire contents of the small and large intestines being liquid, with marked distention. Microscopically there was a marked infiltration of the submucosa with leucocytes and lymphocytes, also hyperemia. The mucosa showed areas of sloughing and necrosis of the tips of the villi (postmortem). The Peyer's patches were frequently enlarged.

The kidneys were dark purplish red and showed areas of hemorrhagic infiltration with cloudy swelling of the cortex.

The liver was somewhat enlarged, but usually it showed no marked microscopic changes.

The heart was usually dilated and filled with a blood clot, the muscle being thin and flabby. Microscopically there were small areas of parenchymatous degeneration.

Blood cultures were uniformly sterile; urine cultures showed no proteus; *B. coli* was present twice; and spore formers, once or twice.

#### ANTITOXIN PRODUCTION

Five normal rabbits were selected and their serums tested for ability to protect other rabbits. Five cubic centimeters of their serum were allowed to stand at room temperature with 10 c.c. of the toxin onehalf hour before injection intravenously. (Table II.)

Four of the 5 died quickly. The fifth survived after severe illness.

To 3 of the rabbits from which serum had been tested, 7 weekly doses of eleven-day filtrate, increasing from 0.2 c.c. to 6 c.c. were given intramuscularly without apparent ill effects or loss of weight except slight diarrhea for two

TABLE II

EFFECT OF INJECTION OF TOXIN FROM ELEVEN-DAY BROTH CULTURES WITH SERUM FROM NONIMMUNIZED RABBITS

| SERUM FROM RABBIT NUMBER | C.C. OF SERUM | C.C. OF TOXIN | RESULT                                                                             |
|--------------------------|---------------|---------------|------------------------------------------------------------------------------------|
| 1                        | 10            | 10            | Very weak; lay on side; temperature, 105° F. Loss of 680 gm. in 14 days. Recovery. |
| 2                        | 4½            | 9             | Died in 48 hours.                                                                  |
| 3                        | 5             | 12            | Found dead in less than 18 hours.                                                  |
| 4                        | 4             | 7½            | Found dead in less than 18 hours.                                                  |
| 5                        | 5             | 11            | Died with marked emaciation in 8 days.                                             |

days in one, following the fourth dose. The fourth rabbit was given similar increasing doses of broth.

Eleven days after the last dose, the serum was tested again. (Table III.)

TABLE III

EFFECTS OF INJECTION OF ELEVEN-DAY TOXIN WITH SERUM FROM RABBITS WHICH HAD BEEN IMMUNIZED BY SEVEN INTRAMUSCULAR WEEKLY DOSES OF TOXIN, THE CONTROL RECEIVING THE EQUIVALENT DOSE OF BROTH

| SERUM FROM RABBITS | C.C. OF SERUM | C.C. OF TOXIN | RESULT                                                                                                    |
|--------------------|---------------|---------------|-----------------------------------------------------------------------------------------------------------|
| Immune, A          | 4             | 10            | Slight diarrhea next day. Lost 200 gm. in 5 days. Well. Never weak.                                       |
| Immune, B          | 4             | 10            | Died in less than 18 hours.                                                                               |
| Immune, B          | 4             | 10            | Lost 260 gm. in 3 days. Snuffles appeared on the third day. Recovery.                                     |
| Immune, C          | 4             | 10            | Slight diarrhea. Lost 240 gm. in 3 days. Not very ill. Began to gain on third day. 1590 gm. on fifth day. |
| Control            | 4             | 10            | Died in less than 18 hours.                                                                               |
| Control            | 4             | 10            | Died on third day, severe diarrhea.                                                                       |

In 3 rabbits more than three times the lethal amount of toxin was neutralized by 4 c.c. of immune serum. In one case 4 c.c. of immune serum failed to protect, but a second rabbit, after receiving the same amount of this anti-serum and filtrate showed only moderate loss of weight with slight diarrhea and recovered.

Four cubic centimeters of the serum from the rabbit injected with broth failed to protect in 2 rabbits, one dying in twenty-four hours, the other in three days.

#### SKIN TESTS

The results of intradermal tests on rabbits show first that susceptibility to the filtrate is comparatively low. However, of 13 rabbits tested, all but one reacted positively to the undiluted toxin, and in this one the reaction area was almost up to the 1 cm. diameter taken as a minimum standard for a positive reaction.

Seven of the 13 rabbits were positive in dilution of 1:10, the reaction being less than 2 cm. in diameter in all but one.

Two of the 4 rabbits, given increasing intramuscular injections of the filtrates weekly for six weeks, reacted negatively to undiluted toxin after

having been positive to 1:10 dilution before immunization. The other two showed definite diminution in the intensity of the skin reaction.

Two rabbits which had been given transient bladder infections with the organism two months before reacted positively to a 1:10 dilution in the skin test.

To test the toxicity of the filtrate for man, preparations as described were diluted with normal salt solution, and the reaction of the skin to intradermal injections of 0.1 c.c. of various dilutions was observed eighteen and twenty-four hours later. A reaction area measuring  $1 \times 1$  cm. or more, from eighteen to twenty-four hours after injection was considered a positive test.

In susceptible persons, redness appeared at the site of injection shortly after the test was made, fading in an hour or less. About four or six hours later pinkness the size of the welt was present and gradually increased in size and intensity to its maximum which might be reached in six or eighteen hours; it usually began to fade after about twenty-four hours and disappeared in from forty-eight to seventy-two hours. With well-marked reactions the color on the second day was brownish red fading to brown. If the reaction was fairly intense, from slight to moderate induration was present with elevation at the site of injection.

Freshly filtered and diluted toxin was found to give a positive test in 1 of 2 adults and 4 of 13 children (from three to six years old) in a dilution of 1:255,000. The reactions were faint. This solution was found to deteriorate rapidly, two weeks later giving a negative test in the previously positive persons.

Tests made at intervals during four and one-half months with 1:5,000 dilutions were positive. The last tests were about two-thirds the size of the first, indicating a slight deterioration at ice box temperature.

The solution, heated for one hour at  $100^{\circ}$  C., was used as control and was found to give only slight redness at site of injection, or negative tests. Test solution heated for two hours at  $100^{\circ}$  C. showed no redness at site of injection in twelve cases reacting positively to the unheated toxin and faint reactions a centimeter or less in diameter in two cases.

Strains of the organism kept on blood agar for three months retained the ability to produce a highly toxic filtrate. One such filtrate (eleven-day culture) gave a positive skin reaction in one and a negative skin test in 2 adults in dilution of 1:50,000. This filtrate two months later gave a negative test in the susceptible person in dilution 1:20,000, but was positive in dilution 1:10,000 and strongly positive in dilution 1:5,000. The skin test area was slightly larger in the case of this filtrate than in that from the freshly isolated strain.

#### CONCLUSIONS

*B. proteus* produces a toxin, the toxicity of the filtrate increasing with the age of the culture. Toxin production is about four times as great in gelatin as in peptone broth. The sterile toxin injected intravenously into rabbits causes diarrhea, loss of weight, and death.



Neutralization of this toxin in vitro by blood serum from rabbits immunized with it indicates that it is a soluble exotoxin capable of producing an antitoxin in rabbits.

Toxin solutions deteriorate on standing at ice box temperature and more rapidly at higher dilutions. The toxin is fairly stable in undiluted form. *B. proteus* cultures kept on artificial mediums for three months do not lose the ability to produce toxin.

Skin tests made with various dilutions of the toxin indicate that man is much more susceptible than the rabbit.

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PROFESSIONAL BUILDING

## COCAINE POISONING AS INFLUENCED BY DIETS\*

### PRELIMINARY REPORT

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THE influence of diets, especially with alkalinizing and acidifying tendency, has been previously studied, particularly in connection with phosphorus poisoning. We have been interested in altering the acid-base balance of rabbits as a basis for studies in connection with seasonal diets and climatic changes. Hoping that we may find, in certain drugs, reagents that might help us in this study, we undertook feeding experiments upon rabbits, intended to alter their acid-base equilibrium, and then injected in these animals toxic doses of cocaine. The results have been sufficiently striking to make this preliminary report seem desirable.

We have used in these experiments various diets that will promote a prevalence of acids or bases in animals in the normal physiologic limits without causing any noticeable pathologic changes in them.

Forty-eight experiments were performed on thirty rabbits. These experiments were divided into three series of sixteen each. In each series, before the performance of an experiment, the animals were kept on certain diets for a period of three weeks. In the first series, the animals were kept on oatmeal

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and water; in the second series, on carrots; and, in the third, on oatmeal, water, and carrots. During the dieting the urine of the animals was tested daily for its reaction (Folin's method). From the second week on the urine of rabbits of the first series of experiments showed distinct and stable acid reaction (from 14 to 60); the urine of the animals of the second series was persistently alkaline; and that of the third series, slightly alkaline or neutral. In the third series of experiments, the same eight rabbits were used twice; the animals of the first and second series of experiments were changed from one diet to another. Ten rabbits withstood these experiments and were on both diets, and six succumbed, so that the latter had to be replaced by new animals.

The experiments consisted of injections of cocaine into the marginal ear vein of a rabbit. The injections were performed uniformly, the duration of

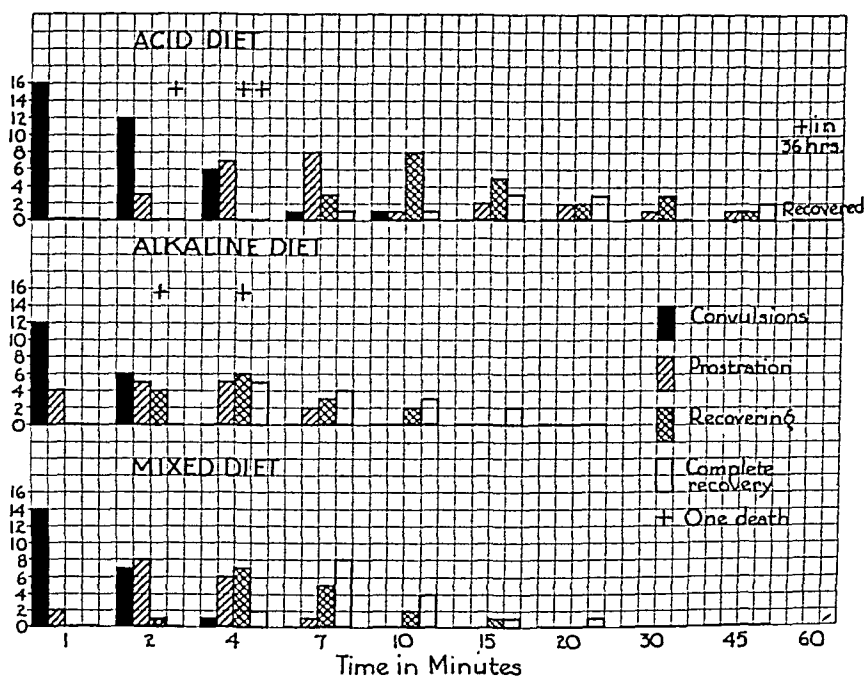


Chart 1.—The perpendicular line shows the number of rabbits. The columns on the horizontal lines are grouped by the time that elapsed from the moment of cocaine injection (1, 2, 4, 7, 10, 15, 20, 30, 45, and 60 minutes) and represent the degree of reactions (see chart) and number of animals. Each group of columns represents the same animals, reacting differently at various time intervals. The animals that died or recovered at certain times of observation are not shown in the following column.

injection being about 10 seconds. The dose of cocaine was 10 mg. per kilo. After injection, the animal was observed as long as symptoms were manifest. The degree of intoxication was recorded by checking the (1) death, (2) convulsions, (3) complete prostration without convulsions, (4) recovering (crawling, lifting head), and (5) recovery. Before the injection of cocaine a certain amount of blood was withdrawn from the ear vein and tested for its hydrogen ion concentration. The average  $P_H$  of the blood of the rabbits on acid diet was 7.301, on alkaline diet 7.426, and mixed diet 7.423, which showed that the rabbits in our experiments were within the limits of the normal acid-base balance.

The results are presented in Chart 1.

The described observations permit us to arrive at the following conclusions:

1. The animals on alkaline-forming diet and on mixed diet reacted to the injection of cocaine, more or less similarly; though the animals on the alkaline diet reacted more vigorously, since we recorded two deaths in this series.

2. The animals on acid-forming diet were definitely more sensitive to cocaine poisoning. Here were recorded four deaths; and, in general, the toxic effects of the cocaine were definitely greater and recovery was considerably delayed.

3. The observations presented suggest the investigation of a number of other powerful drugs in connection with their action upon the animal organism in various states of acid-base balance. Only then would it be permissible to speculate upon the reason for the difference noted.

4. It may be possible, that, in acute cocaine poisoning, the intravenous injection of alkali might be of benefit for the poisoned individual; and experiments upon animals, to test this question, are called for.

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## NASOPHARYNGEAL FLORA AND SOME REMARKS AS TO THEIR RELATION TO COMMON COLDS\*

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THE common cold is generally defined as an acute or subacute infection of the mucosa of the nasopharynx, often invading the sinuses and occasionally the middle ear through the eustachian tube. At times, it may also extend through the trachea, larynx, and bronchial tree. Furthermore, it may leave a chronic condition locally or elsewhere.

Although the common cold is considered the mildest and least serious of all known common diseases, yet it is most frequent, annoying, and depressing; and it often results in the lowering of resistance of the individual, thus making him prey to more serious diseases. Generally speaking, no one is exempt from attacks of colds. Some have occasional attacks, others frequent attacks. One may have mild infection, while others have severe ones. In either case, it lessens the efficiency of the worker, resulting in great total loss economically.

A tremendous amount of experimental and research work is carried on concerning common colds, both in this country and abroad. Medical periodicals have published voluminous articles on etiology, pathology, and treatment of this condition. Unfortunately, it seems we are still wandering in the dark and very little has come to light which points to a definite conclusion about this common ailment.

In reviewing the literature on this subject, within the last few years, one

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finds that quite a few well-outlined and definite studies have been carried on by various workers. I will quote briefly the conclusions of some of the papers:

From their cultural studies of nose and throat of 105 adults and children, Webster and Hughes<sup>1</sup> isolated pneumococci in 80 per cent of cases, and out of 500 pneumococcic strains 97 per cent were serologically specific. Successive cultures from given carriers, with rare exceptions, had a constant serologic type and were similar in type of colonies, morphology, virulence for mice, and in other biologic characteristics. They conclude also that the incidence of pneumococci in all individuals studied undergoes seasonal variation paralleling that of coryza and sore throat in the same person. Third, some patients were pneumococcus-free; some were transient carriers; some periodic, and others chronic carriers. The data suggest that these differences are due to variations in the host resistance.

According to the observations made by Webster and Clow<sup>2</sup> of the association of pneumococci, *Hemophilus influenzae* and *Streptococcus hemolyticus* with coryza, pharyngitis, and sinusitis in man, they came to the conclusion that persons free of those organisms mentioned above are, in general, free of coryza, sore throats, influenza, and sinus diseases. Also they found that persons who are occasional or periodic carriers of these organisms may be negative on tests carried out during long healthy periods, but that they generally become positive during or following attacks and subsequently become negative again with returning health.

Third, their data suggest that persons who are chronic carriers show during these illnesses an increase in the number of organisms in the throat and wider distribution of these organisms in the air passages. These observations bring out the intimate relationship between pathogenes of upper respiratory diseases and their symptoms, but they do not disclose the actual nature of this relationship.

In their experimental studies of the common cold, Dochez, Shibley, and Mills<sup>3</sup> state that in both spontaneous and in experimentally induced colds in anthropoid apes, the most significant change that occurs is the increase of activity on the part of the potential pathogenes habitually present in the throat flora. Coincidentally, with the appearance of symptoms, they found that pneumococci, *Streptococcus hemolyticus* and Pfeiffer's bacillus developed in greatly increased numbers and spread over wide areas of nasopharyngeal membranes. These organisms become, after a time, conspicuous in the nose where they are seldom or never present under normal conditions. This has also been observed in human beings. Furthermore, they emphasize the fact that these organisms are secondary invaders and that the most significant factor in common cold pathogenesis seems to be their capacity to incite greater activity on the part of the more dangerous pathogenic organisms which infect the upper respiratory tract.

D. Thompson and R. Thompson,<sup>4</sup> in an extensive article, stress several points on common colds; that the common cold is not a definite single disease, but a group of diseases caused by several different species of organisms and other factors. According to their classification, colds are divided into three groups: (1) bacterial; (2) allergic; and (3) virus colds.

The bacterial flora of the healthy respiratory tract is plentiful and in great variety but has not been thoroughly worked out and classified. The streptococcus is the most common organism of any virulence generally found; next in frequency of occurrence are the pneumococci and *B. influenzae*.

The following are observations made in infants over a period of two years by Kneeland and Dawes.<sup>5</sup> In two severe winter outbreaks of respiratory infection, a parallel rise in carriers of pathogenic organisms was noted. The first autumn outbreak of colds seemed to provide the dissemination of pathogenic organisms. The relationship of colds to the more severe infections was roughly reciprocal. The peak of the carrier rate was reached in March. It seemed that *Hemophilus influenzae* was more predominant in the first year, and pneumococci, in the second year of the study.

Recent studies of filtrable viruses as etiologic factors of colds, by Dochez, Shibley and Mills<sup>6</sup> suggest that the contagious cold in human beings is caused by an invisible and unculturable, filtrable agent which, in all likelihood, belongs to the group of so-called submicroscopic viruses. Colds can be transmitted successfully, they state, from man to chimpanzee and from man to man by means of Berkefeld filtrates of nasal washings obtained from individuals suffering with spontaneous colds, more especially during the period of rising incidence of infection in the community. This experimental cold resembles in all respects those spontaneously contracted in the natural environment.

Cultivation of the virus of the common cold by Powell and Clowes<sup>7</sup> implies, according to their work, that 80 per cent of subjects used experimentally contracted inoculated colds. No subjects were used except those who had no history of colds for two months and showed records of two or three colds per year. They were inoculated during that period of the year when colds were not prevalent. This verified the conclusion of Dochez that the virus of the common cold is easily cultivated in an appropriately prepared chick embryo and is readily transmitted to human subjects.

Burkey and Freese<sup>8</sup> lay claim to isolating a gram-negative filtrable anaerobic organism in 92 out of 114 examinations of individuals in widely separated communities. Positive cultures were obtained from persons with or without colds both during the acute and the later stages of the disease. The strains which were cultivated were prevalent in normal throats as well as in the nasopharynxes of persons suffering from colds at the time. More satisfactory results were obtained in filtering the nasopharyngeal washings through a Berkefeld V-filter. This series of examinations was too small to lead to definite conclusions. The results indicate, however, the presence of these organisms in material from the mouth as well as the pharynx.

In studies concerning the prevention and treatment of colds with vaccine, Murray<sup>9</sup> compared a group of 85 persons who were 100 per cent susceptible to colds with a group of uninoculated controls, within a period of six months covering the seasons of prevalence of colds. Figures and clinical experience with a mixed catarrhal vaccine in that clinic have shown that vaccine therapy offers as much, if not more, than any other preventive measure that has been tried against colds. It is well worth the while to try this type of preventive measure in persons susceptible to colds.

Three years of experience with stock vaccine in colds and in the prevention of respiratory disorders, according to one observer in the literature, indicated that the value of stock vaccines varies with the individual subjected to the therapy.<sup>10</sup>

The purpose of our study was:

(a) To isolate the organisms in the nose and throat of individuals having frequent colds, during the acute attack.

(b) To study in a like manner a number of individuals without colds, or relatively immune to colds.

(c) To carry through parallel cultures of these organisms in hormone broth and in the blood of the subject.

(d) To prepare vaccines from these growths found in the autogenous blood and to attempt the immunization of the subjects with these vaccines.

(e) To attempt, incidentally, to determine the effects of tonsillectomy on the frequency of colds.

These studies were carried out on patients visiting the Out-Patient Department of the Montefiore Hospital. Cultures were taken from 200 patients. From these, 100 were chosen as controls because they had had no colds for a long period of time. The other 100 patients gave histories of frequent colds throughout the year. A few of these complained of regular seasonal colds. In this series 82 were males and 118 females; 64 had had tonsillectomy; 136 had tonsils intact.

The culture technic was carried out as follows: The throat of each patient was rinsed with sterile salt solution. A cotton swab was then rubbed against the pharyngeal wall and a second swab was contaminated in the nostril. Special care was taken not to contaminate the swab by touching the floor of the mouth. These swabs were placed in nutrient broth, incubated for from 24 to 48 hours, and subcultured on blood agar plates. Parallel with these broth cultures, swabs treated as described above were placed in from 3 to 5 c.c. of fresh whole blood obtained from the patient's vein. After 24 hours of incubation, a drop of this blood was plated and the colonies resulting were identified.

Finally, vaccines were prepared from the organisms isolated in the autogenous blood cultures and the patients who were highly susceptible to colds were inoculated. The technic of preparation of the vaccine was as follows: A 48-hour growth of the organism isolated in the autogenous blood medium in each case and subcultured on hormone broth was treated chemically, utilizing saturated trichresol solution, giving a final dilution of 0.5 per cent trichresol in the vaccine. In 48 hours this was tested for sterility. The vaccine was administered in a starting dose of 1 ml twice a week and was progressively increased to a maximum of 15 ml twice a week. With this technic, the patient received the benefit of the activity of both the bacteria and its elaborated toxin in broth media. Patients who were highly susceptible to colds were inoculated with vaccines prepared according to the method just mentioned. The number of patients that received regular treatments thus far was only seventeen, and it is hardly fair to draw final conclusions. Ten

of seventeen showed relief from colds thus far, and the rest had marked improvement; the frequency and severity were markedly diminished. Final report will be given at the end of treatments administered to a hundred or more cases.

Results of the studies:

(a) The cultures in hormone broth generally showed a mixed growth of various organisms. Staphylococci, streptococci, pneumococci, *Micrococcus catarrhalis*, and unidentified gram-positive and gram-negative cocci and bacilli.

(b) Cultures in autogenous blood media generally showed a single organism, but occasionally more than one.

Incidence of various bacteria in broth culture and in autogenous blood cultures.

TABLE I  
TWO HUNDRED CULTURES FROM NASOPHARYNGES IN AUTOGENOUS BLOOD

| ORGANISM                       | NUMBER | PERCENTAGE |
|--------------------------------|--------|------------|
| Staphylococci                  | 126    | 63         |
| Streptococci                   | 78     | 39         |
| Pneumococci                    | 64     | 32         |
| <i>Micrococcus catarrhalis</i> | 8      | 4          |
| Gram-positive bacilli          | 12     | 6          |
| Gram-negative bacilli          | 4      | 2          |
| Sterile                        | 40     | 20         |

TABLE II  
ONE HUNDRED CULTURES FROM NASOPHARYNGES OF PATIENTS FREE FROM COLDS

| ORGANISM                       | BROTH MEDIUM | AUTOGENOUS BLOOD |
|--------------------------------|--------------|------------------|
| Staphylococci                  | 55           | 51               |
| Streptococci                   | 53           | 41               |
| Pneumococci                    | 52           | 38               |
| <i>Micrococcus catarrhalis</i> | 45           | 4                |
| Gram-positive bacilli          | 10           | 4                |
| Gram-negative bacilli          | 45           | 5                |
| Sterile                        | 0            | 10               |

TABLE III  
ONE HUNDRED CULTURES FROM NASOPHARYNGES OF PATIENTS SUSCEPTIBLE TO COLDS

| ORGANISM                       | BROTH MEDIUM | AUTOGENOUS BLOOD |
|--------------------------------|--------------|------------------|
| Staphylococci                  | 64           | 63               |
| Streptococci                   | 50           | 39               |
| Pneumococci                    | 38           | 31               |
| <i>Micrococcus catarrhalis</i> | 22           | 4                |
| Gram-positive bacilli          | 11           | 6                |
| Gram-negative bacilli          | 5            | 2                |
| Sterile                        | 0            | 21               |

Analysis: Tables II and III may be compared. The former applies to the 100 controls who were, as stated, free from colds. The latter is descriptive of the flora found in 100 patients who were susceptible to colds. The highest number of cultures showed staphylococci, considering the entire 200 cultures. Next in the same group we found streptococci and pneumococci to be most numerous, in the order named. It should be noted also that 20 per cent of the cultures carried out on autogenous blood remained sterile. From the comparative study of these tables, it is easily seen that those organisms grown on broth and unidentified failed to grow in the autogenous blood medium.

According to the theory propounded by Dr. Meyer Solis-Cohen and coworkers<sup>11</sup> an organism is pathogenic to the individual only if it is grown in his blood; and failure to do so indicates resistance in the individual to the organism. His findings, further, have been confirmed by others; notably Bull and Bartual,<sup>12</sup> Black, Fowler and Pierce,<sup>13</sup> Smiley,<sup>14</sup> and T. Matsunami and J. A. Kolmer.<sup>15</sup>

#### CONCLUSIONS

1. Individuals who are free from colds, and those who have frequent colds show no notable difference from each other in their nasopharyngeal bacterial flora.

2. The resistance of the individual is probably a more important factor in susceptibility to colds than is the nasopharyngeal bacterial flora.

3. Vaccines prepared from cultures of organisms of the nasopharyngeal flora on autogenous blood media (pathogen selective) appear to have given relief from the tendency to the common cold in 59 per cent of the cases.

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# LABORATORY METHODS

## A SIMPLIFIED MICRODETERMINATION OF CHOLESTEROL IN WHOLE BLOOD, SERUM, AND PLASMA\*

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THE basis of all colorimetric methods for the determination of cholesterol is the Liebermann-Burchard reaction, which is conducted on an anhydrous chloroform solution of the blood cholesterol. Bloor<sup>1</sup> obtains this by extracting the cholesterol with hot alcohol ether, evaporating the solvent and dissolving the residue in chloroform. This method, especially in the micromodification of Sackett<sup>2</sup> is most widely used in clinical laboratories. Other methods dry the blood on plaster of Paris at 100° C. (Myers and Wardell<sup>3</sup>) or on filter paper in air (Ling<sup>4</sup> and Leiboff<sup>5</sup>) and extract with hot chloroform in special continuous extraction apparatus.

The use of heat occasionally produces a brownish off-color tinge in the unknown, which renders colorimetric comparison difficult. We therefore tried to determine if the extraction of the cholesterol may be effected at room temperature without the use of complicated extraction apparatus. Preliminary experiments showed that the cholesterol is quantitatively extracted by chloroform from blood dried on filter paper in two hours at room temperature. Therefore, the following procedure was adopted for the determination of cholesterol in blood.

### PROCEDURE

On an ordinary 7 cm. filter paper, preferably ashless, deposit 0.2 c.c. of oxalated blood, serum, or plasma. Work the paper around until the liquid is distributed fairly evenly over the surface and then suspend it by means of a thread or wire strung through an unstained area in an incubator at from 35° to 40° C. After from twenty to thirty minutes the blood is completely dried. Roll the paper up compactly; fold into quarters and deposit it in the bottom of an ordinary 6 inch × 1 inch test tube. Into the tube, pipette 10 c.c. of chloroform; cork, and set aside for at least two hours. During this period the chloroform should completely cover the folded filter paper. In this manner a quantitative extraction of cholesterol from dried blood, serum, or plasma is effected without the use of heat, thus avoiding the occasional appearance of an off-color. Now, pipette 5 c.c. of the chloroform solution into an ordinary test tube. Into a similar tube place 5 c.c. of the working cholesterol standard.<sup>†</sup>

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†Stock Cholesterol Standard: Dissolve 1 gm. C.P. cholesterol in chloroform to make 100 c.c. of solution.

Working Cholesterol Standard: Dilute 5 c.c. of the stock cholesterol standard with chloroform to make 1000 c.c. of solution; 1 c.c. contains 0.95 mg. cholesterol. Both standards keep indefinitely in a refrigerator.

To each tube add 2 c.c. of acetic anhydride and 0.2 c.c. of concentrated sulphuric acid. Stopper, mix by inversion and allow to stand for fifteen minutes in a cool, dark place. Compare in the colorimeter.

Comparison:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 250 = \text{mg. cholesterol per 100 c.c. blood.}$$

Set unknown at 25 mm. and compare. The reading of the standard multiplied by ten gives the milligrams of cholesterol in 100 c.c. of whole blood, serum, or plasma.

This method is especially suited for use in clinical laboratories where the determination of cholesterol is conducted as part of a routine. The blood may be dried and allowed to extract with chloroform while the remainder of the routine is carried on. The actual working time per determination is from four to five minutes.

This procedure was conducted on 125 specimens of blood, serum, and plasma ranging in cholesterol content from 76 to 585 mg. per 100 c.c. and checked by the Bloor-Sackett method. In all cases, the results obtained agreed within 9 mg. per 100 c.c. with an average difference of 3.55 mg.

#### SUMMARY

Blood is dried in air on filter paper, extracted with chloroform at room temperature for at least two hours, and the Liebermann-Burchard reaction is conducted on the extract.

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## EXAMINATION FOR TUBERCLE BACILLI<sup>2</sup>

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KOERTH and Hibbard<sup>1</sup> have recently reported a comparison of methods for staining tubercle bacilli, based on the use of three stains. They claimed better than 25 per cent advantage for the Spengler stain over the Ziehl-Neelsen method. The Schulte-Tigges method, though nearly as efficient as the Spengler by their test, was ruled out as more difficult and more subject to errors in technic.

We have examined one hundred sputums chosen at random, except that they had been reported negative or contained less than six bacilli with the Ziehl-Neelsen technic. Fifty were twenty-four-hour specimens, the other fifty being collections over a period of three days.

When the results were being tabulated, it was found that seventy-one patients were represented, covering all stages of the disease. A large number had never given a positive sputum by direct smear, nor had guinea-pig inoculations proved a diagnosis of tuberculosis on many of them.

A five-minute time standard was used. By "positive" is meant that six or more bacilli were found during this period. In a "negative," no bacilli were found during a five-minute search. If less than six bacilli were seen during the five-minute interval, the number is given.

The results are shown in the following table:

| STAIN         | NEGATIVE | POSITIVE, MORE<br>THAN 6<br>BACILLI | LESS THAN 6<br>BACILLI† | NUMBER OF<br>SLIDES SHOWING<br>BACILLI |
|---------------|----------|-------------------------------------|-------------------------|----------------------------------------|
| Ziehl-Neelsen | 95       | 0                                   | 5                       | 5                                      |
| Spengler      | 85       | 10‡                                 | 5                       | 15                                     |

†Not recognized as positive by some authorities.

‡Five of these positives were those specimens where less than six bacilli were found by the Ziehl-Neelsen method. The Spengler simply increased the number.

The conclusion is drawn in favor of the Spengler method. We have also found the method satisfactory in tissue staining.

A second study was made on the comparative value of two concentration methods, the hydrogen peroxide<sup>2</sup> and the antiformin method.<sup>3</sup>

Here, too, the series was short. Examinations were made of some twenty cases which had given negatives in from one examination to examinations covering several months. Some had never given a positive.

Except when guinea pigs were to be inoculated, the tests were made on sputum sterilized by autoclave. Controls of known positives, both sterile and unsterile, were used by both methods. Tubercle bacilli found in the glands

<sup>2</sup>From the Grasslands Hospital, Valhalla, New York.  
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and spleen of an inoculated guinea pig proved that the organism remained viable after being subjected to the hydrogen peroxide treatment.

The two procedures checked closely, one more positive slide being found by the peroxide concentration than by the antiformin method. As this procedure was much less time consuming, making it possible to report the result of an examination on the day the specimen was received, it was chosen as our routine. In a group of two hundred specimens (all of which had been negative at the examination immediately preceding) treated by this method and centrifuged fifteen to thirty minutes at fairly high speed, we were able to report thirty-one positives and the presence of a few bacilli (less than six) in four other cases. This made thirty-five concentrations, or 17.5 per cent, showing organisms when from one to many previous examinations had been negative.

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CROTON HEIGHTS, YORKTOWN HEIGHTS

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## HEMOGLOBIN AND BLOOD CELL RELATIONS AS DETERMINED BY IRON AND OXYGEN CAPACITY METHODS\*

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IN A recent article Haden<sup>3</sup> has summarized the various methods employed for the determination of hemoglobin and the present state of knowledge regarding the normal hemoglobin content of blood. He points out a certain lack of agreement in the three largest series of determinations of hemoglobin in normal men and women. The oxygen capacity hemoglobin method of Van Slyke was the basis of comparison in the series quoted, namely, those of Osgood,<sup>5</sup> Wintrobe and Morell,<sup>8</sup> and Haden.<sup>4</sup> The absence of any large series of hemoglobin determinations in normal individuals in which iron determinations are used as the basic method is noted by Haden and also pointed out as desirable by Peters and Van Slyke.<sup>6</sup>

We are, accordingly, reporting hematologic studies on a number of patients and normal individuals of both sexes, in which the hemoglobin determinations were based both on the oxygen capacity and the iron content of blood.

Most of the individuals examined lived in or near St. Louis, although some were medical students who had spent the greater part of their lives in other localities in this country. Twenty-three normal men and thirteen normal women were studied. In addition observations were made on thirty patients ill with various conditions.

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Blood was drawn shortly before noon, with minimal stasis, into a large syringe from which it was delivered into a 25 c.c. volumetric flask containing exactly 3 c.c. of 1.6 per cent sodium oxalate solution. All work was done on the mixed dilution so obtained. A 1 c.c. to 200 c.c. dilution with Hayem's

TABLE I  
HEMOGLOBIN AND CELL RELATIONS IN NORMAL MEN

| HEMOGLOBIN AND CELL RELATIONS |                                     |              |              |                      |                                        |                        |                    |                     |             |                      |              |                  |  |
|-------------------------------|-------------------------------------|--------------|--------------|----------------------|----------------------------------------|------------------------|--------------------|---------------------|-------------|----------------------|--------------|------------------|--|
| DESIGNATION OF INDIVIDUAL     | HEMOGLOBIN GRAMS PER 100 C.C. BLOOD |              |              |                      | ERYTHROCYTE COUNT, MILLIONS PER C.M.M. | HEMOGLOBIN COEFFICIENT | PIGMENT/CELL RATIO | HEMOGLOBIN PER CENT | COLOR INDEX | CELL VOLUME PER CENT | VOLUME INDEX | SATURATION INDEX |  |
|                               | OXYGEN CAPACITY                     | PROTEIN IRON | ACID HEMATIN | THREE-METHOD AVERAGE |                                        |                        |                    |                     |             |                      |              |                  |  |
| 1                             | 18.30                               | 17.76        | 18.03        | 18.03                | 5.280                                  | 17.07                  | 3.43               | 119                 | 1.12        | 48.7                 | 1.07         | 1.05             |  |
| 2                             | 18.18                               | 17.48        | 17.71        | 17.79                | 5.400                                  | 16.48                  | 3.29               | 118                 | 1.09        | 48.8                 | 1.05         | 1.05             |  |
| 3                             | 18.00                               | 17.54        | 17.50        | 17.68                | 5.320                                  | 16.62                  | 3.34               | 117                 | 1.10        |                      |              |                  |  |
| 4                             | 17.68                               | 17.75        | 17.28        | 17.57                | 5.920                                  | 14.83                  | 2.97               | 116                 | 0.98        | 48.0                 | 0.94         | 1.05             |  |
| 5                             | 17.60                               | 17.61        | 17.10        | 17.44                | 5.420                                  | 16.11                  | 3.24               | 115                 | 1.06        | 46.6                 | 1.00         | 1.06             |  |
| 6                             | 17.10                               | 17.10        | 17.70        | 17.30                | 5.480                                  | 15.78                  | 3.16               | 114                 | 1.04        | 49.5                 | 1.05         | 0.99             |  |
| 7                             | 17.65                               | 17.30        | 16.88        | 17.28                | 5.600                                  | 15.42                  | 3.09               | 114                 | 1.12        | 47.5                 | 0.98         | 1.04             |  |
| 8                             | 17.30                               | 16.90        | 17.25        | 17.15                | 4.910                                  | 17.45                  | 3.49               | 113                 | 1.22        | 47.5                 | 1.12         | 1.03             |  |
| 9                             | 17.10                               | 16.89        | 16.63        | 16.87                | 5.380                                  | 15.69                  | 3.13               | 111                 | 1.03        | 46.2                 | 1.00         | 1.04             |  |
| 10                            | 17.00                               | 16.82        | 16.59        | 16.80                | 5.270                                  | 15.93                  | 3.19               | 111                 | 1.05        |                      |              |                  |  |
| 11                            | 16.41                               | 16.66        | 16.88        | 16.65                | 4.740                                  | 17.56                  | 3.51               | 110                 | 1.16        | 47.0                 | 1.15         | 1.01             |  |
| 12                            | 16.70                               | 16.10        | 16.68        | 16.49                | 5.760                                  | 14.31                  | 2.86               | 109                 | 1.04        | 50.0                 | 1.01         | 0.94             |  |
| 13                            | 16.50                               | 16.40        | 16.48        | 16.46                | 5.560                                  | 14.79                  | 2.96               | 109                 | 1.08        | 45.5                 | 0.95         | 1.03             |  |
| 14                            | 16.58                               | 16.58        | 16.20        | 16.45                | 4.920                                  | 16.75                  | 3.34               | 109                 | 1.11        |                      |              |                  |  |
| 15                            | 16.48                               | 16.20        | 16.38        | 16.35                | 4.780                                  | 17.11                  | 3.42               | 108                 | 1.13        | 44.1                 | 1.09         | 1.04             |  |
| 16                            | 16.54                               | 16.21        | 15.75        | 16.17                | 5.100                                  | 15.84                  | 3.17               | 107                 | 1.05        | 45.8                 | 1.04         | 1.01             |  |
| 17                            | 16.80                               | 15.92        | 15.53        | 16.08                | 5.200                                  | 15.45                  | 3.09               | 106                 | 1.02        | 46.6                 | 0.99         | 1.07             |  |
| 18                            | 16.04                               | 16.06        | 15.47        | 15.86                | 5.060                                  | 15.67                  | 3.14               | 105                 | 1.04        | 45.4                 | 1.04         | 1.00             |  |
| 19                            | 15.67                               | 15.84        | 15.80        | 15.77                | 5.020                                  | 15.70                  | 3.14               | 104                 | 1.04        | 43.8                 | 1.02         | 1.02             |  |
| 20                            | 15.60                               | 15.51        | 16.11        | 15.74                | 5.226                                  | 15.06                  | 3.02               | 104                 | 0.99        | 44.0                 | 0.98         | 1.01             |  |
| 21                            | 15.58                               | 15.58        | 15.96        | 15.71                | 5.360                                  | 14.65                  | 2.93               | 104                 | 0.97        | 46.1                 | 1.00         | 0.97             |  |
| 22                            | 15.58                               | 15.00        | 15.10        | 15.23                | 5.500                                  | 13.84                  | 2.77               | 101                 | 0.95        | 45.5                 | 0.97         | 0.96             |  |
| 23                            | 15.25                               | 15.00        | 15.10        | 15.12                | 5.560                                  | 13.60                  | 2.72               | 100                 | 0.94        | 44.9                 | 0.94         | 0.96             |  |

TABLE II  
HEMOGLOBIN AND CELL RELATIONS IN NORMAL WOMEN

| DESIGNATION OF INDIVIDUAL | HEMOGLOBIN GRAMS PER 100 C.C. BLOOD |              |              |                      | ERYTHROCYTE COUNT, MILLIONS PER C.M.M. | HEMOGLOBIN COEFFICIENT | PIGMENT/CELL RATIO | HEMOGLOBIN PER CENT | COLOR INDEX | CELL VOLUME PER CENT | VOLUME INDEX | SATURATION INDEX |
|---------------------------|-------------------------------------|--------------|--------------|----------------------|----------------------------------------|------------------------|--------------------|---------------------|-------------|----------------------|--------------|------------------|
|                           | OXYGEN CAPACITY                     | PROTEIN IRON | ACID HEMATIN | THREE-METHOD AVERAGE |                                        |                        |                    |                     |             |                      |              |                  |
| 24                        | 15.90                               | 15.24        | 15.02        | 15.39                | 4.788                                  | 16.07                  | 3.22               | 102.0               | 1.08        | 43.0                 | 1.09         | 1.08             |
| 25                        | 15.50                               | 15.14        | 15.30        | 15.31                | 4.920                                  | 15.57                  | 3.16               | 101.0               | 1.07        | 43.2                 | 1.02         | 1.07             |
| 26                        | 15.33                               | 15.16        | 15.41        | 15.30                | 4.900                                  | 15.61                  | 3.19               | 101.0               | 1.03        | 42.7                 | 1.01         | 1.04             |
| 27                        | 14.53                               | 14.85        | 15.30        | 14.89                | 4.840                                  | 15.39                  | 3.08               | 98.4                | 1.02        | 42.0                 | 1.01         | 1.04             |
| 28                        | 14.29                               | 14.08        | 14.64        | 14.34                | 4.860                                  | 14.75                  | 2.95               | 94.8                | 0.98        | 40.9                 | 0.98         | 1.03             |
| 29                        | 14.51                               | 13.98        | 13.98        | 14.16                | 4.680                                  | 15.12                  | 3.03               | 93.5                | 0.96        | 41.0                 | 1.01         | 1.01             |
| 30                        | 14.40                               | 13.88        | 14.17        | 14.15                | 4.280                                  | 16.54                  | 3.31               | 93.5                | 1.09        | 40.0                 | 1.09         | 1.07             |
| 31                        | 14.20                               | 13.88        | 13.82        | 13.97                | 4.680                                  | 14.93                  | 2.98               | 92.3                | 0.99        | 40.8                 | 1.02         | 1.04             |
| 32                        | 14.20                               | 13.20        | 13.88        | 13.83                | 4.870                                  | 14.20                  | 2.84               | 91.4                | 0.94        | 38.2                 | 0.91         | 1.06             |
| 33                        | 13.40                               | 12.81        | 13.19        | 13.13                | 5.030                                  | 13.00                  | 2.61               | 86.8                | 0.86        | 39.3                 | 0.91         | 0.95             |
| 34                        | 12.50                               | 12.90        | 13.00        | 12.80                | 2.834                                  | 13.25                  | 2.65               | 84.6                | 0.87        | 37.5                 | 0.90         | 1.00             |
| 35                        | 12.80                               | 12.52        | 12.90        | 12.74                | 5.228                                  | 12.12                  | 2.44               | 84.1                | 0.80        | 39.8                 | 0.88         | 0.87             |
| 36                        | 12.40                               | 12.00        | 12.15        | 12.18                | 4.240                                  | 14.36                  | 2.87               | 80.2                | 0.95        | 36.4                 | 0.99         | 0.96             |

TABLE III  
OBSERVATIONS ON PATIENTS

| DESIGNATION<br>OR<br>DIAGNOSIS                                                 | HEMOGLOBIN<br>GRAMS PER 100 C.C. BLOOD |              |              |                         | ERYTHROCYTE COUNT,<br>MILLIONS PER C.M.M. | HEMOGLOBIN<br>COEFFICIENT | PIGMENT/CELL<br>RATIO | HEMOGLOBIN<br>PER CENT | COLOR INDEX | CELL VOLUME<br>PER CENT | VOLUME INDEX | SATURATION INDEX |
|--------------------------------------------------------------------------------|----------------------------------------|--------------|--------------|-------------------------|-------------------------------------------|---------------------------|-----------------------|------------------------|-------------|-------------------------|--------------|------------------|
|                                                                                | OXYGEN<br>CAPACITY                     | PROTEIN IRON | ACID HEMATIN | THREE-METHOD<br>AVERAGE |                                           |                           |                       |                        |             |                         |              |                  |
| 72532<br>Familial Hemolytic<br>Icterus                                         | 17.40                                  | 17.08        | 17.35        | 17.28                   | 5.530                                     | 15.4                      | 3.09                  | 113.0                  | 1.02        | 46.6                    | 0.98         | 1.05             |
| 72442<br>Lethargic Encephal-<br>itis with Vomiting.<br>Free HCl present        | 17.15                                  | 16.80        | 17.18        | 17.04                   | 5.590                                     | 15.2                      | 3.05                  | 112.0                  | 1.01        | 48.8                    | 1.01         | 0.99             |
| 71874<br>Amenorrhea with Gen-<br>ital Hypoplasia.<br>Depressive Psycho-<br>sis | 16.70                                  | 16.20        | 16.20        | 16.37                   | 4.880                                     | 16.3                      | 3.36                  | 108.0                  | 1.11        | 45.8                    | 1.02         | 0.99             |
| 72317<br>Gastroenteritis                                                       | 15.90                                  | 16.01        | 15.80        | 15.90                   | 5.320                                     | 15.0                      | 3.17                  | 105.0                  | 0.99        | 44.3                    | 0.98         | 1.02             |
| 71963<br>Aortic Stenosis                                                       | 15.85                                  | 15.85        | 15.00        | 15.57                   | 5.084                                     | 15.2                      | 3.08                  | 102.0                  | 1.00        | 45.4                    | 1.03         | 0.97             |
| 71991<br>Duodenal Ulcer. En-<br>teroptosis                                     | 15.02                                  | 15.23        | 15.23        | 15.16                   | 5.620                                     | 13.5                      | 2.70                  | 110.0                  | 0.89        | 44.3                    | 0.92         | 0.97             |
| 70861<br>Epilepsy                                                              | 14.50                                  | 14.81        | 14.59        | 14.63                   | 4.540                                     | 16.0                      | 3.24                  | 96.6                   | 1.06        | 42.0                    | 1.08         | 0.99             |
| 72131<br>Glomerular Nephritis                                                  | 14.64                                  | 14.40        | 14.53        | 14.52                   | 4.442                                     | 16.3                      | 3.19                  | 96.0                   | 1.08        | 39.7                    | 1.04         | 1.04             |
| 71985<br>Toxic Goiter                                                          | 15.00                                  | 13.88        | 14.00        | 14.29                   | 5.170                                     | 13.9                      | 2.76                  | 94.2                   | 0.91        | 41.0                    | 0.92         | 0.99             |
| 71960<br>Cholecystitis and<br>Cholelithiasis                                   | 14.13                                  | 13.72        | 13.84        | 13.90                   | 4.960                                     | 14.0                      | 2.80                  | 91.8                   | 0.93        | 43.2                    | 1.01         | 0.92             |
| 71881<br>Lobar Pneumonia                                                       | 13.85                                  | 13.01        | 12.93        | 13.26                   | 4.270                                     | 15.6                      | 3.11                  | 87.5                   | 1.02        | 38.6                    | 1.05         | 0.97             |
| 71812<br>Hypertension, Myo-<br>carditis, Asthma                                | 13.30                                  | 13.10        | 13.18        | 13.19                   | 4.950                                     | 13.2                      | 2.67                  | 87.2                   | 0.88        | 41.3                    | 0.97         | 0.91             |
| 72803<br>Cholecystitis and<br>Cholelithiasis                                   | 13.50                                  | 12.60        | 13.10        | 13.07                   | 4.180                                     | 15.6                      | 3.13                  | 86.3                   | 1.04        | 40.7                    | 1.13         | 0.91             |
| 72029<br>Toxic Goiter                                                          | 12.81                                  | 12.63        | 12.73        | 12.72                   | 4.600                                     | 13.8                      | 2.74                  | 84.0                   | 0.92        | 34.0                    | 0.86         | 1.06             |
| 71841<br>Cholecystitis with<br>Cholelithiasis. Ach-<br>lorhydria               | 12.59                                  | 12.44        | 12.70        | 12.58                   | 4.110                                     | 15.3                      | 3.06                  | 82.9                   | 1.01        | 36.9                    | 1.04         | 0.96             |
| 71956<br>Migraine                                                              | 12.92                                  | 12.23        | 12.08        | 12.41                   | 4.600                                     | 13.5                      | 2.70                  | 82.0                   | 0.89        | 38.6                    | 0.98         | 0.91             |
| 72478<br>Pelvic Peritonitis                                                    | 12.30                                  | 12.13        | 12.13        | 12.19                   | 4.890                                     | 12.5                      | 2.49                  | 80.5                   | 0.92        | 38.2                    | 0.91         | 0.91             |
| 68797<br>Pernicious Anemia                                                     | 12.20                                  | 12.30        | 11.75        | 12.08                   | 3.290                                     | 19.4                      | 3.67                  | 79.7                   | 1.25        |                         |              |                  |
| 72053<br>Bronchiectasis                                                        | 12.32                                  | 11.48        | 11.66        | 11.82                   | 4.220                                     | 14.0                      | 2.86                  | 78.2                   | 0.93        | 35.6                    | 0.98         | 0.94             |
| 72032<br>Dietary Deficiency,<br>Protein Depriva-<br>tion, Achlorhydria         | 12.15                                  | 11.64        | 11.47        | 11.75                   | 3.480                                     | 16.9                      | 3.38                  | 77.6                   | 1.11        | 34.1                    | 1.13         | 0.98             |

TABLE III—CONT'D

| DESIGNATION<br>OR<br>DIAGNOSIS                               | HEMOGLOBIN<br>GRAMS PER 100 C.C. BLOOD |              |              |                         | ERYTHROCYTE COUNT,<br>MILLIONS PER C.M.M. | HEMOGLOBIN<br>COEFFICIENT | PIGMENT/CELL<br>RATIO | HEMOGLOBIN<br>PER CENT | COLOR INDEX | CELL VOLUME<br>PER CENT | VOLUME INDEX | SATURATION INDEX |
|--------------------------------------------------------------|----------------------------------------|--------------|--------------|-------------------------|-------------------------------------------|---------------------------|-----------------------|------------------------|-------------|-------------------------|--------------|------------------|
|                                                              | OXYGEN<br>CAPACITY                     | PROTEIN IRON | ACID HEMATIN | THREE-METHOD<br>AVERAGE |                                           |                           |                       |                        |             |                         |              |                  |
| 71989<br>Syphilis, Glaucoma                                  | 11.88                                  | 11.64        | 11.70        | 11.74                   | 3.960                                     | 14.9                      | 2.96                  | 77.5                   | 0.98        |                         |              |                  |
| 72218<br>Pernicious Anemia.<br>Posterosclerotic<br>Sclerosis | 10.34                                  | 10.38        | 10.57        | 10.43                   | 2.676                                     | 19.6                      | 3.90                  | 69.0                   | 1.29        | 32.0                    | 1.38         | 0.93             |
| 72522<br>Bacterial Endocard-<br>itis                         | 10.40                                  | 10.28        | 10.47        | 10.38                   | 3.540                                     | 14.7                      | 2.93                  | 68.6                   | 0.97        | 32.4                    | 1.06         | 0.92             |
| 72577<br>Acute Rheumatic<br>Fever                            | 10.20                                  | 9.42         | 9.60         | 9.74                    | 4.080                                     | 11.9                      | 2.39                  | 64.2                   | 0.79        | 30.0                    | 0.86         | 0.92             |
| 72119<br>Chronic Myelogenous<br>Leucemia                     | 9.70                                   | 9.52         | 9.42         | 9.55                    | 3.090                                     | 15.4                      | 3.09                  | 63.0                   | 1.02        |                         |              |                  |
| 71816<br>Neurosyphilis. Thera-<br>peutic Malaria             | 9.68                                   | 9.09         | 8.99         | 9.25                    | 3.400                                     | 13.6                      | 2.72                  | 61.0                   | 0.90        | 25.0                    | 0.85         | 1.05             |
| 72504<br>Hypotension. Addi-<br>son's Disease                 | 9.10                                   | 8.28         | 8.63         | 8.67                    | 3.380                                     | 12.8                      | 2.56                  | 57.2                   | 0.85        | 28.4                    | 0.98         | 0.87             |
| 72109<br>Hypertrophy of Pros-<br>tate. Pyelonephritis        | 7.07                                   | 7.15         | 7.67         | 7.29                    | 2.310                                     | 15.8                      | 3.16                  | 48.1                   | 1.04        | 21.6                    | 1.08         | 0.96             |
| 71780<br>Pernicious Anemia                                   | 4.56                                   | 5.12         | 5.15         | 4.94                    | 1.310                                     | 18.9                      | 3.77                  | 32.6                   | 1.25        |                         |              |                  |
| 72230<br>Bacterial Endocar-<br>ditis                         | 2.97                                   | 3.03         | 3.29         | 3.10                    | 1.324                                     | 11.7                      | 2.34                  | 20.5                   | 0.78        | 11.4                    | 0.98         | 0.79             |

solution was made for enumeration of blood cells. The count reported represents the average of four to eight fills of the counting chamber. Bureau of Standards equipment was used. Hematocrit determinations were made in duplicate after saturating with oxygen in sedimentation tubes and centrifuging to constant values.

The oxygen capacity method used was that of Van Slyke and Neil.<sup>7</sup> Hemoglobin determinations based on iron were made by two separate technics recently devised in this laboratory.<sup>1</sup> In one hemoglobin was calculated from a colorimetric estimation of protein iron as Prussian blue, and in the other by comparing the acid hematin color with an acid hematin standard of known iron content.

The results of our observations appear in Tables I, II, and III. The hemoglobin as determined by protein iron throughout the series is found to be slightly lower than that found by oxygen capacity. In general, however, the agreement between the two methods is excellent. We have, therefore, used the average of the three methods for comparison with the hemoglobin content of blood as found by other observers. This comparison will be seen





in Table IV. The figures of Osgood and Haskins, Wintrobe, and Haden are quoted from the article of Haden to which we have already referred. It will be noted that the figures obtained by averaging the results of these three series of observations are in more general agreement with our results than are those of any one individual series of observations. Perhaps the most important figure in the table is that termed "hemoglobin coefficient," indicating grams of hemoglobin per 100 c.c. blood when the erythrocyte count is 5,000,000 per c. mm. In regard to this figure the results of Haden are in much closer agreement with our figures than are those of Osgood and Haskins and Wintrobe.

The figure designated as "Pigment-Cell Ratio" is obtained by dividing the grams of hemoglobin per 100 c.c. blood by the millions of erythrocytes per c. mm. of blood. The figure obtained actually represents the milligrams of hemoglobin present in 100,000,000 erythrocytes. Could the precedent be established of using this figure in place of the conventional color index, it would encourage the practice of reporting hemoglobin in terms of grams per 100 c.c., and the color index, if desired, could be obtained approximately by dividing this figure by three as suggested by Cullen.<sup>2</sup> However, it seems obvious to us that the practice of reporting hemoglobin per cent and color index might well be abandoned for the reason that the normal hemoglobin value is much higher in men than in women. Calculations from an arbitrary standard are, therefore, necessary although undesirable. In the present study hemoglobin per cent was calculated from the mean of the average hemoglobin coefficients for normal men and women.

The average erythrocyte count for normal men was found to be 5.29 millions per c. mm. The average erythrocyte count for normal women was 4.62 millions.

The average hemoglobin value for men was 16.60 gm., with variations between 15.12 and 18.03 gm. For women the average hemoglobin value was 14.0 gm., ranging between 12.18 and 15.39 gm.

The average hemoglobin coefficient for men was 15.7 gm.; for women, it was 14.7 gm.

The average cell volume per cent for normal men was 46.57 per cent. For normal women it averaged 40.37 per cent. The mean of the averages of the two sexes was 43.47 per cent. The mean of the average of the two sexes per 5,000,000 erythrocyte count was 43.1 per cent.

The average saturation index of normal men was observed to be 1.017. That of normal women was likewise 1.017.

The pigment-cell ratio averaged 3.15 for the normal males and 2.95 for the normal females. The mean ratio was, therefore, 3.05. The normal limits for this figure were found to be between 2.44 and 3.51. It can be noted that a high figure was found in the two cases of pernicious anemia observed, namely, 3.67 and 3.90 and a low figure in the achromic anemias as shown by cases 72577 and 72230, namely, 2.39 and 2.34. Hence this figure can be used as well as the color index to determine the type of anemia.

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## A COMPARISON OF METHODS FOR THE DETERMINATION OF URIC ACID IN HUMAN, BOVINE, AND AVIAN BLOODS\*†

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NUMEROUS methods for the determination of uric acid in human blood have been developed, the chief motive being to incorporate the higher degree of accuracy of the isolation methods with the greater simplicity and ease of manipulation of the direct methods. While several of these methods have given satisfactory results on human blood, few of them have been applied to the blood of other animals. Since in this laboratory we have been especially interested in the analysis of bovine and avian (chicken) bloods, the present study was undertaken to ascertain the most satisfactory method for the determination of uric acid in these bloods.

### DISCUSSION OF DATA

In Table I data are presented to show the results of analyses of human, bovine, and avian bloods, using the Folin-Wu laked blood filtrate<sup>15</sup> and the Folin unlaked blood filtrate.<sup>12</sup> The methods of Folin<sup>11, 13</sup> of Folin as modified by Bulmer, Eagles and Hunter,<sup>5</sup> of Benedict,<sup>2</sup> and of Brown<sup>7</sup> were used with each of these filtrates, each method being applied to the filtrate directly and to the uric acid isolated as the silver salt from each filtrate. Thus twelve methods were used with each sample of blood.

The data in Table I show that in most cases the isolation methods give lower results than the direct methods. This fact is more evident in the case of laked than in the case of unlaked blood filtrates, the difference being especially pronounced in the case of laked filtrates from bovine blood. Direct and

\*From the Department of Agricultural and Biological Chemistry of The Pennsylvania State College, State College of Pennsylvania.

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TABLE I  
SHOWING A COMPARISON OF THE URIC ACID CONTENT OF HUMAN, BOVINE, AND AVIAN BLOODS BY THE METHODS OF FOLIN, BENERICT, AND BROWN ON LAKED AND UNLAKED BLOOD FILTRATES

| SPEC. NO.                           | LAKED BLOOD |           |      |          |           |          | UNLAKED BLOOD |           |      |        |           |      |          |           |      |        |           |          |
|-------------------------------------|-------------|-----------|------|----------|-----------|----------|---------------|-----------|------|--------|-----------|------|----------|-----------|------|--------|-----------|----------|
|                                     | FOLIN       |           |      | BENEDICT |           |          | BROWN         |           |      | FOLIN  |           |      | BENEDICT |           |      | BROWN  |           |          |
|                                     | DIRECT      | ISOLATION | *    | DIRECT   | ISOLATION | *        | DIRECT        | ISOLATION | *    | DIRECT | ISOLATION | *    | DIRECT   | ISOLATION | *    | DIRECT | ISOLATION | *        |
| MG. URIC ACID PER 100 G.C. OF BLOOD |             |           |      |          |           |          |               |           |      |        |           |      |          |           |      |        |           |          |
| HUMAN                               |             |           |      |          |           |          |               |           |      |        |           |      |          |           |      |        |           |          |
| 1                                   | 4.2         | 3.2       | 3.4  | 2.9      | 3.7       | 2.9      | 3.1           | 2.4       | 2.8  | 2.8    | 2.8       | 3.5  | 2.8      | 2.8       | 2.8  | 3.5    | 3.2       | 3.2      |
| 2                                   | 5.2         | 4.1       | 4.5  | 3.2      | 5.6       | 4.5      | 5.4           | 4.1       | 4.8  | 4.8    | 4.8       | 5.9  | 4.8      | 4.8       | 4.8  | 5.9    | 4.6       | 4.6      |
| 3                                   | 4.6         | 3.3       | 3.6  | 3.0      | 3.5       | 3.7      | 3.4           | 3.5       | 2.7  | 2.7    | 2.7       | 5.2  | 2.7      | 2.6       | 2.6  | 5.2    | 4.1       | 4.1      |
| 4                                   | 5.7         | 4.9       | 4.8  | 4.5      | 4.5       | 3.9      | 4.6           | 2.8       | 4.6  | 4.6    | 4.6       | 5.8  | 4.6      | 4.7       | 4.7  | 5.8    | 3.8       | 3.8      |
| 5                                   | 5.3         | 4.6       | 4.2  | 3.6      | 5.4       | 4.7      | 4.4           | 4.6       | 4.1  | 4.1    | 4.1       | 4.3  | 4.2      | 4.2       | 4.2  | 4.3    | 4.2       | 4.2      |
| 6                                   | 3.9         | 3.0       | 3.1  | 3.0      | 4.6       | 3.6      | 3.3           | 3.2       | 3.2  | 3.2    | 3.2       | 3.8  | 3.2      | 3.2       | 3.2  | 3.8    | 4.0       | 4.0      |
| 7                                   | 4.3         | 3.4       | 3.6  | 3.1      | 4.6       | 3.7      | 3.4           | 3.4       | 2.9  | 2.9    | 2.9       | 3.4  | 3.4      | 3.4       | 3.4  | 3.8    | 4.0       | 4.0      |
| Average                             | 4.74        | 3.76      | 3.89 | 3.32     | 4.56      | 3.86     | 3.94          | 3.42      | 3.58 | 3.58   | 3.58      | 4.83 | 3.69     | 3.69      | 3.69 | 4.83   | 4.13      | 4.13     |
| BOVINE                              |             |           |      |          |           |          |               |           |      |        |           |      |          |           |      |        |           |          |
| 1                                   | 2.2         | 1.5       | 1.4  | 0.7      | 2.0       | no color | 0.5           | 1.3       | 1.4  | 1.4    | 1.4       | 1.2  | 0.3      | 0.3       | 0.3  | 1.2    | no color  | no color |
| 2                                   | 2.5         | 0.7       | 2.6  | 0.6      | 1.4       | 0.4      | 0.6           | 0.3       | 0.7  | 0.7    | 0.7       | 0.9  | 0.6      | 0.6       | 0.6  | 0.9    | no color  | no color |
| 3                                   | 2.5         | 0.6       | 2.2  | 0.8      | 1.8       | 0.6      | 0.5           | 0.5       | 0.8  | 0.8    | 0.8       | 0.6  | 0.5      | 0.5       | 0.5  | 0.6    | no color  | no color |
| 4                                   | 2.7         | 0.7       | 2.0  | 0.6      | 1.7       | 0.7      | 0.8           | 0.5       | 0.9  | 0.9    | 0.9       | 0.6  | 0.5      | 0.5       | 0.5  | 0.6    | no color  | no color |
| 5                                   | 2.2         | 0.7       | 2.0  | 0.6      | 1.7       | 0.7      | 0.5           | 0.4       | 0.7  | 0.7    | 0.7       | 0.6  | 0.6      | 0.6       | 0.6  | 0.6    | no color  | no color |
| 6                                   | 1.9         | 0.9       | 1.9  | 0.6      | 1.7       | 0.7      | 0.5           | 0.4       | 0.7  | 0.7    | 0.7       | 0.6  | 0.6      | 0.6       | 0.6  | 0.6    | no color  | no color |
| Average                             | 2.33        | 0.85      | 2.01 | 0.65     | 1.67      | 0.66     | 0.60          | 0.57      | 0.87 | 0.87   | 0.87      | 0.75 | 0.50     | 0.50      | 0.50 | 0.75   | no color  | no color |
| AVIAN                               |             |           |      |          |           |          |               |           |      |        |           |      |          |           |      |        |           |          |
| 1                                   | 4.6         | 3.0       | 4.1  | 2.8      | 4.4       | lost     | 4.1           | 3.2       | 3.8  | 3.8    | 3.8       | 4.3  | 3.0      | 3.0       | 3.0  | 4.3    | lost      | lost     |
| 2                                   | 4.6         | 3.5       | 4.4  | 3.3      | 5.8       | 4.1      | 5.0           | 3.5       | 4.8  | 4.8    | 4.8       | 5.3  | 3.6      | 3.6       | 3.6  | 5.3    | 4.6       | 4.6      |
| 3                                   | 5.2         | 3.4       | 4.8  | 3.7      | 5.5       | 2.6      | 4.5           | 2.6       | 5.1  | 5.1    | 5.1       | 5.9  | 4.1      | 4.1       | 4.1  | 5.9    | 3.9       | 3.9      |
| 4                                   | 3.9         | 2.1       | 3.7  | 2.0      | 4.6       | 2.6      | 3.0           | 2.6       | 3.1  | 3.1    | 3.1       | 3.5  | 2.9      | 2.9       | 2.9  | 3.5    | 2.7       | 2.7      |
| 5                                   | 4.1         | 3.5       | 4.2  | 3.2      | 4.8       | 2.8      | 3.9           | 2.2       | 3.9  | 3.9    | 3.9       | 5.2  | lost     | lost      | lost | 5.2    | 4.9       | 4.9      |
| 6                                   | 5.1         | 2.3       | 3.9  | 2.3      | 4.6       | 2.8      | 3.1           | 2.2       | 3.1  | 3.1    | 3.1       | 4.8  | 3.2      | 3.2       | 3.2  | 4.8    | 3.2       | 3.2      |
| 7                                   | 5.1         | 5.5       | 5.4  | 4.9      | 8.1       | 7.2      | 7.0           | 6.0       | 5.4  | 5.4    | 5.4       | 8.0  | 5.6      | 5.6       | 5.6  | 8.0    | 7.5       | 7.5      |
| 8                                   | 3.5         | 3.3       | 4.5  | 3.6      | 6.1       | 5.0      | 3.4           | 4.1       | 4.3  | 4.3    | 4.3       | 3.8  | 3.8      | 3.8       | 3.8  | 3.8    | 4.0       | 4.0      |
| 9                                   | 3.1         | 2.6       | 3.9  | 2.6      | 4.3       | 2.8      | 3.0           | 2.6       | 4.2  | 4.2    | 4.2       | 4.7  | 3.4      | 3.4       | 3.4  | 4.7    | 3.3       | 3.3      |
| 10                                  | 2.6         | 1.8       | 2.8  | 1.7      | 4.1       | 1.5      | 2.0           | 1.6       | 2.6  | 2.6    | 2.6       | 3.2  | 1.9      | 1.9       | 1.9  | 3.2    | 2.1       | 2.1      |
| 11                                  | 4.3         | 4.0       | 5.1  | 3.9      | 6.1       | 4.0      | 4.3           | 3.0       | 4.9  | 4.9    | 4.9       | 5.1  | 3.7      | 3.7       | 3.7  | 5.1    | 4.5       | 4.5      |
| Average                             | 4.08        | 3.18      | 4.25 | 3.09     | 5.31      | 3.69     | 3.93          | 3.27      | 4.11 | 4.11   | 4.11      | 5.07 | 3.42     | 3.42      | 3.42 | 5.07   | 4.07      | 4.07     |

All figures are averages of duplicate determinations.

• Five cubic centimeters of silver lactate solution were used in these cases as suggested by Bulmer, Baglos, and Hunter.<sup>5</sup>

isolation procedures agree more closely on unlaked than on laked filtrates. All isolation procedures give results which agree fairly well.

With regard to the great differences in uric acid values obtained by the direct and isolation procedures on bovine blood, the work of Benedict and his coworkers<sup>9, 10, 17</sup> should be mentioned. They have isolated from the erythrocytes of bovine blood a combined form of uric acid which is not precipitated by silver-magnesia mixture. The low values obtained with the isolation method and with the direct methods on unlaked filtrates from bovine blood might be explained on this basis, since combined uric acid would not be precipitated by the silver lactate in the isolation procedure and, being in the corpuscles, would not be present in unlaked blood filtrates.

Bulmer, Eagles, and Hunter<sup>8, 16</sup> ascribed the discrepancy between the direct and isolation methods to a substance "X" which they isolated from animal bloods, including bovine blood, and which they called a simple pyrimidine nucleoside. This compound is precipitated by silver lactate but is not liberated by acid sodium chloride, so that it would not appear in the isolation procedure.

Almost simultaneously, Benedict and his coworkers<sup>1, 3, 4</sup> isolated from pig and human bloods an interfering substance "thiasine" or thioneine, as it was later called. They claimed that this compound is not the same as substance "X," and that it causes high values in the direct method.

From the averages given in Table I it appears that for human blood Benedict's direct method applied to Folin's unlaked blood filtrate gives results more closely agreeing with the isolation procedure than any of the other methods. In the case of bovine blood, Folin's direct method applied to an unlaked blood filtrate gives results agreeing most closely with the isolation procedure. In the case of avian blood, the agreement between the direct and isolation procedures on an unlaked filtrate is about the same for Folin's as for Benedict's method.

Brown's method, with one exception, gives higher results on human and avian bloods than the other methods. In the case of bovine blood, there was no color produced when Brown's isolation procedure was applied to the unlaked filtrate. When Brown's direct method was applied to a laked blood filtrate, the results were lower than the direct methods of Folin and of Benedict.

In 1931 Benedict<sup>5</sup> suggested the use of tungstomolybdic acid for the preparation of laked blood filtrates. He<sup>6</sup> also suggested a modification of the isolation procedure. In Table II Benedict's laked blood filtrate is compared with Folin's laked and unlaked filtrates from human, bovine, and avian bloods. Folin's direct<sup>13</sup> and isolation<sup>8</sup> methods are applied to all three filtrates, and Benedict's direct<sup>2</sup> and new isolation<sup>6</sup> procedures are used on Benedict's laked filtrates.

From the averages in Table II it appears that in the case of human blood results by all the isolation methods agree closely. Of the direct methods that of Folin, used with his unlaked filtrate, gives results which agree most closely with the results obtained by the isolation procedure. It should be noted, however, that in Table I, with an unlaked filtrate, results by Benedict's direct method agreed more closely with the isolation procedure than did Folin's.

TABLE II

SHOWING A COMPARISON OF THE URIC ACID CONTENT OF HUMAN, BOVINE, AND AVIAN BLOODS BY THE DIRECT AND ISOLATION METHODS OF FOLIN AND BENEDICT ON UNLAKED AND TWO TYPES OF LAKED BLOOD FILTRATES

| SPLE. NO. | LAKED-FOLIN                         |            | LAKED-BLOOD-BENEDICT |      |        |            | UNLAKED-FOLIN |            |
|-----------|-------------------------------------|------------|----------------------|------|--------|------------|---------------|------------|
|           | FOLIN                               |            | BENEDICT             |      | FOLIN  |            | FOLIN         |            |
|           | DIRECT                              | ISOLATION* | DIRECT               | NEW  | DIRECT | ISOLATION* | DIRECT        | ISOLATION* |
| HUMAN     | MG. URIC ACID PER 100 C.C. OF BLOOD |            |                      |      |        |            |               |            |
| 11        | 3.0                                 | 2.7        | 3.3                  | 2.4  |        |            | 3.0           | 2.5        |
| 12        | 3.2                                 | 2.5        | 3.1                  | 2.5  |        |            | 2.5           | 2.7        |
| 13        | 2.8                                 | 2.8        | 2.9                  | 2.0  |        |            | 2.5           | 2.4        |
| 14        | 3.9                                 | 3.2        | 2.7                  | 3.5  |        |            | 3.1           | 2.9        |
| 15        | 2.8                                 | 2.2        | 2.8                  | 2.3  | 2.9    | 2.3        | 2.9           | 2.1        |
| 16        | 3.6                                 | 3.5        | 3.3                  | 3.2  | 3.7    | 3.5        | 2.6           | 3.5        |
| 17        | 2.8                                 | 2.5        | 3.4                  | 2.4  | 3.5    | 2.5        | 2.4           | 2.1        |
| Average   | 3.16                                | 2.77       | 3.07                 | 2.61 | 3.36   | 2.76       | 2.86          | 2.60       |
| BOVINE    |                                     |            |                      |      |        |            |               |            |
| 11        | 1.6                                 | 0.4        | 1.6                  | 0.9  | 1.6    | 0.5        | 0.5           | 0.2        |
| 12        | 1.3                                 | 0.4        | 1.6                  | 0.9  | 1.5    | 0.5        | 0.4           | 0.2        |
| 13        | 1.0                                 | 0.5        | 1.2                  | 0.5  | 1.3    | 0.6        | 0.4           | 0.2        |
| 14        | 1.9                                 | 0.7        | 2.0                  | 1.0  | 1.8    | 0.7        | 0.6           | 0.5        |
| 15        | 1.4                                 | 0.5        | 1.6                  | 0.8  | 1.5    | 0.5        | 0.4           | 0.3        |
| 16        | 1.5                                 | 0.6        | 1.6                  | 1.0  | 1.7    | 0.7        | 0.5           | 0.4        |
| Average   | 1.45                                | 0.51       | 1.60                 | 0.85 | 1.57   | 0.58       | 0.47          | 0.30       |
| AVIAN     |                                     |            |                      |      |        |            |               |            |
| 11        | 2.9                                 | 1.8        | 4.2                  | 2.9  | 3.2    | 1.8        | 2.0           | 1.7        |
| 12        | 2.9                                 | 2.1        | 3.8                  | 3.0  | 3.2    | 1.9        | 2.4           | 2.1        |
| 13        | 3.3                                 | 2.3        | 4.9                  | 2.7  | 3.7    | 2.3        | 2.5           | 2.3        |
| 14        | 3.3                                 | 2.3        | 4.4                  | 2.7  | 3.4    | 2.3        | 2.8           | 2.3        |
| 15        | 3.7                                 | 2.7        | 5.3                  | 2.9  | 4.0    | 2.9        | 2.8           | 2.9        |
| 16        | 3.5                                 | 2.5        | 4.6                  | 2.7  | 3.7    | 2.6        | 2.6           | 2.3        |
| Average   | 3.27                                | 2.28       | 4.55                 | 2.81 | 3.53   | 2.30       | 2.51          | 2.27       |

All figures are averages of duplicate determinations.

\*Five cubic centimeters of silver lactate solution were used in these cases as suggested by Bulmer, Eagles, and Hunter.\*

With bovine blood results by the isolation method do not agree so closely as they do with human blood. Benedict's new method gives the highest values, while Folin's method, applied to his unlaked filtrate, gives the lowest values. In Table I Benedict's isolation procedure applied to Folin's unlaked blood filtrate gives lower results than Folin's isolation procedure.

In the case of avian blood, Benedict's new method gives higher results than any of the other procedures. Folin's method with his unlaked filtrate gives the closest agreement between direct and isolation procedures although in Table I the agreement is nearly as close when Benedict's procedure is used with Folin's unlaked filtrate.

In conclusion, it may be stated that for human and avian bloods, it makes little difference which method is used, provided an isolation procedure is followed. Of the direct methods those of Benedict and of Folin, with Folin's unlaked filtrate, give results agreeing closely with the isolation procedures. For bovine blood Folin's direct method, applied to his unlaked filtrate, agrees most closely with the isolation procedure. The authors have found Folin's method more satisfactory than Benedict's because the use of the urea-cyanide solution eliminates the formation of a troublesome precipitate which is always imminent when Benedict's method is used.

Since this paper was written, Folin<sup>14</sup> has made several revisions in his direct method and in the preparation of his uric acid reagent. While lack of time prevents the repetition of the work here presented, it is felt that Folin's latest technic should be considered in selecting a method for the determination of uric acid in animal bloods.

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#### BLOOD IODINE STUDIES\*

##### III. A SIMPLE RESERVOIR BURET FOR MAKING MICROTITRATIONS

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THE quantitative determination of the minute amount of iodine normally present in the blood and urine requires special precision apparatus. In developing an adequate method it became necessary for Davis<sup>1</sup> to construct a suitable microburet for making the titrations. This was a modification of the original reservoir buret designed by Professor F. C. Koch of the Department of Physiological Chemistry of the University of Chicago. Koch's buret was

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adequate for making the usual microtitrations. It was a decided improvement over the old style of buret in common use.

The titration of the minute amount of iodine present in 10 c.c. of blood, however, required even a more precise buret. It became necessary to employ one accurately graduated to thousandths of a cubic centimeter. We are describing and figuring the simple reservoir buret, Fig. 1, which has finally been developed as a result of this need. This has given satisfaction in the routine daily determinations of the minute amounts of iodine normally present in the blood and urine.

A Kahn 0.2 c.c. graduated pipette was fused to the open end of the tube on the one-stem side of a three-way microbore stopcock. On the two-stem

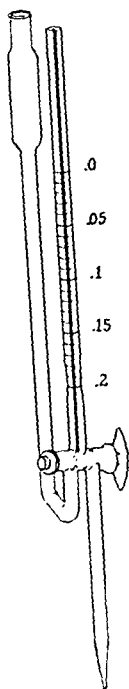


Fig. 1.

side of the three-way stopcock, one tube is drawn to a fine capillary bore and cut 18 cm. from the stopcock, Fig. 1. This length is necessary for titration convenience. The other tube on the two-stem side of the stopcock is bent in a "J" shape. The open end is drawn parallel to the 0.2 c.c. pipette, and about 2 cm. behind it. One centimeter above the level of the zero mark on the 0.2 c.c. pipette a 10 c.c. cylinder-shaped reservoir is fused to the open end of the "J" tube. The accompanying figure of the buret used in this laboratory should be adequate as a design for any competent glass blower to follow in making this apparatus.

The buret described above has been in use in the iodine laboratory for several months. The titrations in the routine daily work here require the measurement of 0.002 c.c. increments of a 0.001 normal sodium thiosulphate

solution. With the above described buret these measurements have been accurately and conveniently carried out.

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### CAGE FOR MICE AND RATS\*

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THIS type of cage has been used by us for the past two years, and we feel that it possesses distinct advantages over other types of cages used heretofore. In our experience some of these advantages consist in the prevention of food and bedding pollution by the segregation of each in a different compartment, the ease and cheapness of construction, the greater accessibility of the animals, the ease of cleaning, the ease of observation of the undisturbed animal and the ease of transportation from place to place. The cage serves well for feeding experiments, breeding purposes, stock animals, and general experimental purposes. It is compact, economical of space, and may be placed on a narrow shelf. Waste cotton is employed for bedding and it is placed in the upper compartment. If so desired, the food may be placed in the upper rather than the lower compartment.

*Dimensions.*—11.25 x 8.5 inches.

*Materials.*—Galvanized hardware cloth ( $\frac{1}{4}$  inch mesh) for body, door, removable floor, and as a guard around opening between the two compartments (to prevent bedding material from falling through into lower compartment); sheet zinc ( $\frac{1}{32}$  inch) for floor of upper compartment, card holder, and to form margin of door opening; sheet metal ( $\frac{1}{16}$  inch) for band at bottom of cage and door catch; heavy wire soldered to edge of mesh door (to provide additional strength and support); water container (6 inch test tube, single hole rubber stopper, glass tubing) fastened by detachable clips to side of body; enamel pan.

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\*From the School of Medicine, University of Alabama.  
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# MAINTAINING WATER AND AIR BALANCE DURING PROLONGED INCUBATION\*

## EXPERIENCES GAINED FROM GROWING TUBERCLE BACILLI.

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DURING the course of years of experience, one frequently stops to glance back and smile at the arbitrariness of formed habits and empiricism which proved exceedingly wasteful of time and material and which condition could easily have been remedied by opportunity for a few moments of proper experimentation and test. Of course, the apology might be offered that the majority

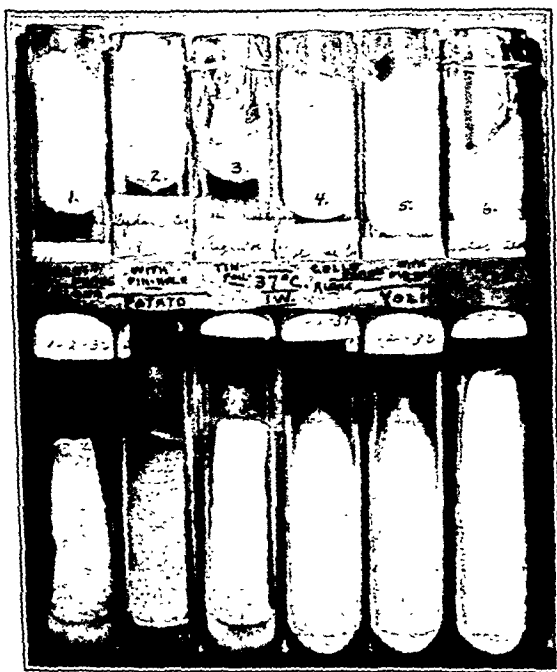


Fig. 1.—The result of capping with cellophane or tin foil one week after incubation at 37° C. upon potato cylinder and inspissated egg yolk medium. Tubes 1 to 3 are the potato medium and Tubes 4 to 6 the egg medium. Tubes 1 and 4 were capped with cellophane over a cotton plug, the cellophane cap over Tubes 2 and 5 was perforated with pin holes and Tubes 3 and 6 were capped with tin foil over a paraffined cotton plug. Note the drying of the top of the medium in the cellophane capped Tubes 1, 2, 4, and 5.

of us have been trained to follow instructions implicitly and not question our textbooks or teachers. In growing tubercle bacilli the technical phases are strikingly different, and it was a stroke of "genius" that led Koch to discover the tubercle bacillus, or rather let us say correctly, in place of genius, his patience and perseverance. It is now fifty years since this discovery was made

\*From the Research Department, National Jewish Hospital at Denver.  
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and bacteriologists in general have failed to recognize fully the technical obstacles encountered in growing tubercle bacilli. Aside from the composition of the medium which has been elaborately studied by us,<sup>1, 2, 3</sup> there are two factors dependent upon technical detail that may spell success or failure in growing these bacilli, the maintenance of the water and air balance. To attempt to maintain the water balance of the medium by any means of supplying water in the incubator or in the cans containing the culture tubes or flasks is obviously futile as only experience will prove, or possibly a little reflection upon mother's bread box, or the storekeeper's unceasing vigil to

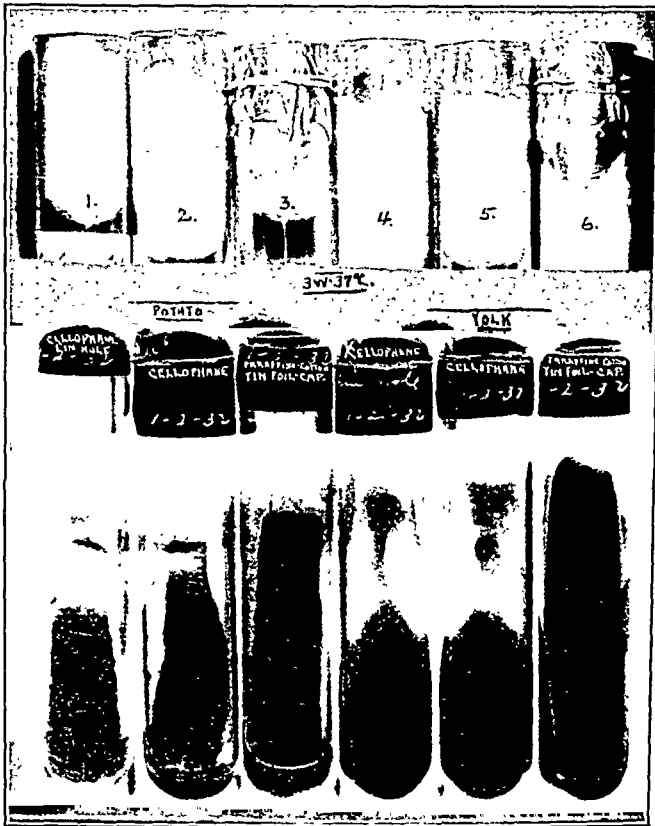


Fig. 2.—The same tubes shown in Fig. 1, after three weeks at 37° C. Note the marked drying of the mediums in Tubes 1, 2, 4, and 5 (cellophane capped) and the evaporation of the condensation water in the bottom of the tubes.

prevent mold disasters, will provide the evidence. And molds extend into the smallest crevices, even through the bacteriologic impervious cotton plug if given time and moisture. Dryness is the enemy of mold and, therefore, it is better to maintain water balance by preventing evaporation; and yet one may ask how to maintain air balance and still prevent evaporation in a dry incubator, particularly in a dry climate of extremely low humidity and over a long period of time. The investigator soon learns that explanations must await facts, and so the experiences to be recorded will be presented as facts which anyone can easily explain on the basis of present knowledge.

It has been the custom of the senior author over many years in growing tubercle bacilli to utilize the method of impregnating cotton plugs or culture tubes with hot paraffin, and then covering the tubes with tin foil or cloth impregnated with paraffin, with the cap secured by a small rubber band to prevent drying of the medium. In practice this method had met the needs apparently satisfactorily, when a trial of cellophane capping was advocated to us as being more easily used and readily applied to the tubes or flasks. The objection raised by us to the use of cellophane was based on our knowledge of the water and moisture permeability of this material, and especially

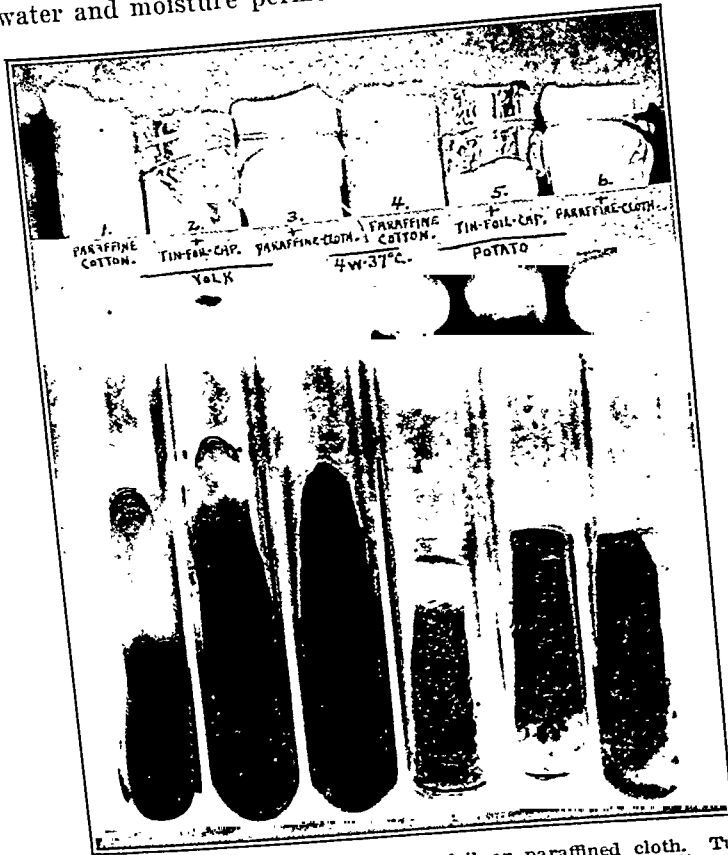


Fig. 3.—A comparison of capping with tin foil or paraffined cloth. Tubes 1, 2, and 3 are inspissated egg yolk medium, Tubes 4, 5, and 6 potato cylinder medium. Tubes 1 and 4 are controls plugged with paraffined cotton. Tubes 2 and 5 are in addition capped with tin foil and Tubes 3 and 6 are capped with paraffined cloth. Note the greater drying of the medium and condensation water in Tubes 1 and 4 uncapped, and the better maintenance of water balance in the paraffin cloth capped Tubes 3 and 6 as compared to the tin foil capped Tubes 2 and 5 after four weeks at 37° C.

because one of us has had intimate knowledge of its use for preevaporation. Those advocating the use of cellophane capping maintained it would save labor and obviate the use of the rubber band as well as paraffining of the cotton plug, and, of course, would make easier the access of air (an erroneous deduction). It was tried with the results shown in Figs. 1 and 2. In commenting on this test it may be noted that very little difference in the rate of drying of the medium occurred regardless of whether the cellophane had been perforated or not.

There are a number of methods which have been variously advocated for maintaining the water balance in culture tubes over relatively long periods of time of incubation, and two of them were cited above, the tin foil capping or paraffin cloth capping, but we are not aware of actual tests recorded in the literature of the relative merits of these two. To us they appeared equally efficient and the tin foil, although more costly, possessed the advantage of being less messy and less bulky. Test, however, proved the paraffin cloth to be more efficient as shown in Fig. 3. In some laboratories it is the

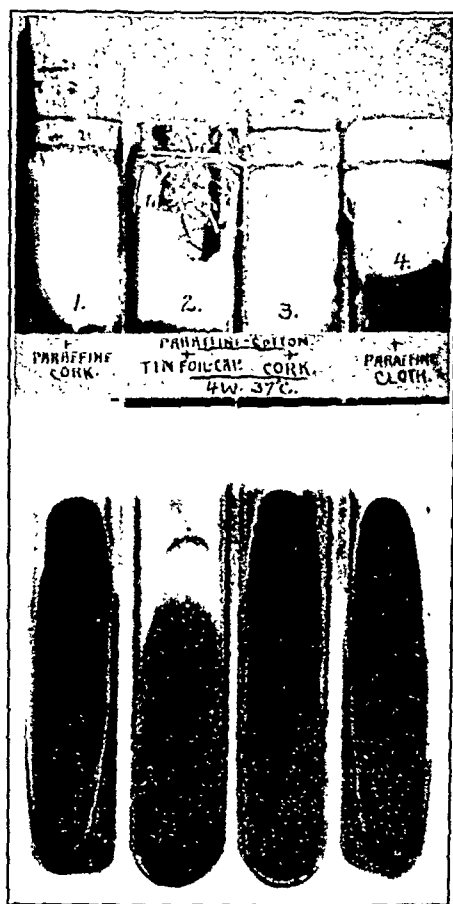


Fig. 4.—A comparison of capping with cork stoppers and tin foil or paraffin cloth after keeping inspissated egg yolk medium for four weeks at 37° C. Note the absence of drying in the tubes stoppered with the plain nonparaffined cork (Tube 3) and with the paraffined cork (Tube 1) as well as with the paraffin cloth cap while Tube 2 tin foil capped showed evidences of drying of the upper portion of this medium at this time.

custom to merely pour a layer of hot paraffin over the cotton plug without attempting to impregnate all the fibers, and then to perforate the paraffin plug with a few pin holes. This method is fairly satisfactory but fails to remove entirely the water absorbing powers of the cotton plug, and each time that the tube is to be opened the plug of paraffin must be removed, which in our hands has proved less satisfactory than other methods, including the paraffin cloth which with the paraffin impregnated cotton plug is more efficient for preventing water evaporation.

Recently one of us<sup>4</sup> described a tissue substrate method for practice, and for simplicity recommended the use of a lightly paraffined cork. It seemed advisable, therefore, to determine the merit of the cork for the purpose of maintaining water and air balance in culture tubes. The maintenance of water balance by the cork placed over the paraffin impregnated cotton plug is well illustrated in Fig. 4.

However, this experiment proved only the adequacy of the cork for maintaining water balance, and our knowledge regarding the use of the cork

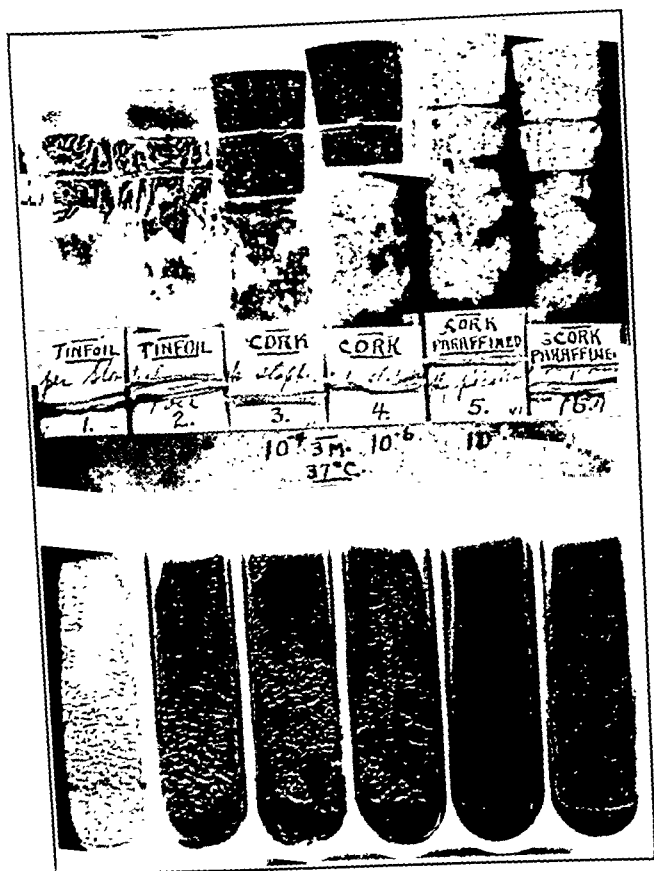


Fig. 5.—The results of capping with tin foil, with the cork stopper (not paraffined) and a paraffined cork stopper upon the growth of small numbers of tubercle bacilli (a human strain) on an inspissated egg yolk medium after three months' incubation at 37° C. All the cotton plugs were impregnated with hot paraffin, and Tubes 1 and 2 were capped with tin foil, Tubes 3 and 4 were stoppered with a plain dry cork, and Tubes 5 and 6 with a paraffined cork inserted fairly tightly. Note that the growth of the bacilli in the plain corked Tubes (3 and 4) is equal to that in the tin foil capped tubes (1 and 2) in both the 0.000,1 mg. plantings (Tubes 1 and 3) and in the 0.000,001 mg. plantings (Tubes 2 and 4), while in both plantings the growth was retarded in the tubes stoppered with the paraffined corks (Tubes 5 and 6). The latter finding is readily understood on the basis of the relative imperviousness of the paraffined cork to air especially when tightly inserted into the tube.

as a stopper for effervescing and fermenting beverages made us doubt its value in maintaining air balance. Manometric tests assured us that the dry cork was readily pervious to air, but that the cork heavily impregnated with paraffin and tightly inserted into a tube was almost completely impervious to air and was, therefore, unsuited to our use for growing tubercle bacilli.

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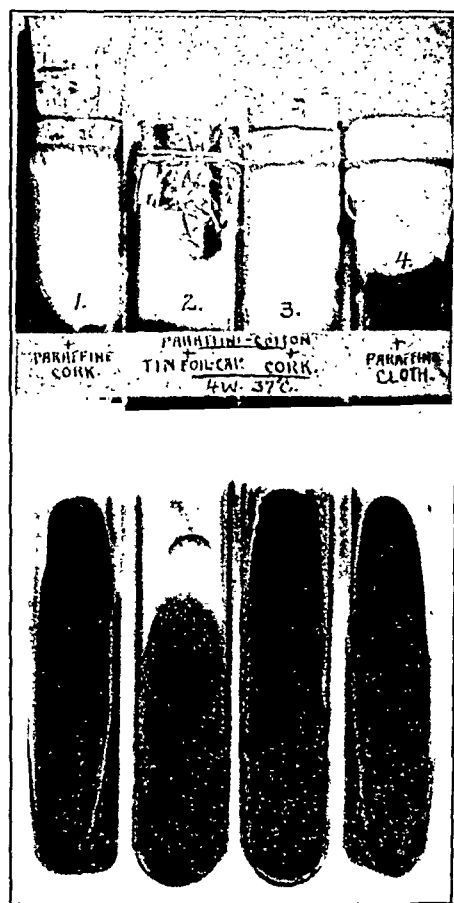


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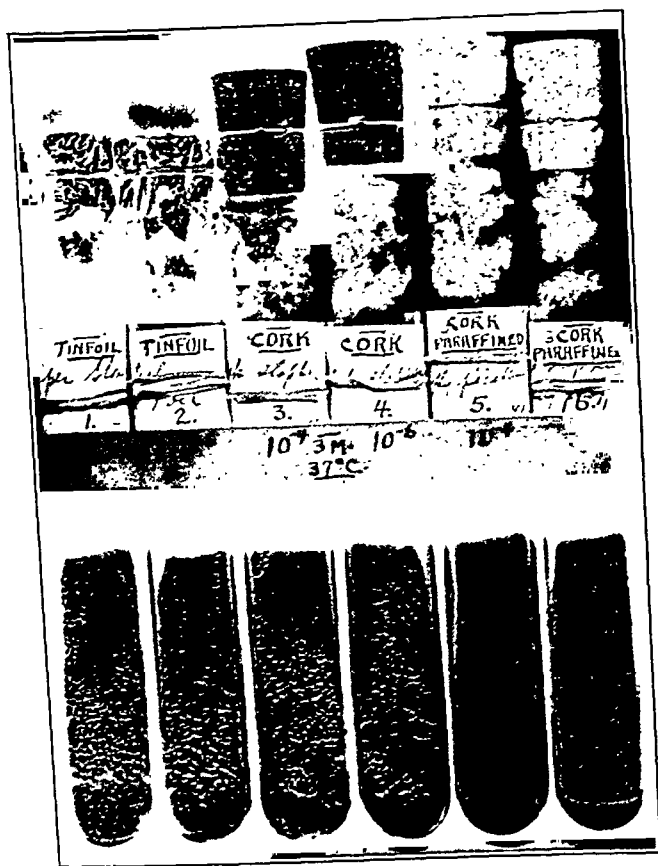


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If the cork was only lightly impregnated with paraffin and was only loosely inserted into the tube, it would not prevent air access in sufficient amount for growing tubercle bacilli. It is well also to remember that the water wet cork is far less pervious to air than the dry cork. To further test the value of the dry cork, it was given a thorough trial in culture tests with tubercle bacilli in graded seedings (see Fig. 5), and proved sufficiently valuable both from an economic and efficiency standpoint so that it is now used in all our culture tube work in place of tin foil or paraffin cloth, being inserted above the cotton plug which has been impregnated completely but lightly with hot paraffin. The corks can be repeatedly used and can be freed from paraffin, if desired, by washing in gasoline or some other readily volatile paraffin solvent, after which they may be air dried and, finally, to free from traces of the solvent, may be placed in the dry sterilizer for an hour or two.

The efficiency of the paraffining procedure in maintaining water and air balance in culture flasks is well illustrated by a report just presented by us<sup>7</sup> in which we found that tubercle bacilli remained viable for over twelve years after growing in nursing bottles containing broth, stoppered with a paraffined cotton plug, and covered with paraffined cloth. Some of these bottles lost as little as one-quarter to one-half ounce of liquid out of an original three ounces placed in the flask twelve years previously.

In conclusion, it may be stated that the problem of maintaining water and air balance in culture tubes and culture receptacles appears to be one of preventing the conduction of moisture from these receptacles by avoiding the use of moisture absorbent materials, or by satisfactorily impregnating these with a completely nonabsorbent and water impervious material, such as paraffin. It also appears that this can be attained without appreciable influence upon adequate gaseous exchange, especially sufficient to maintain conditions required for the multiplication of slow growing microorganisms, such as the tubercle bacillus.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**LIVER, Silver Nitrate Reaction and Its Applicability to the Diagnosis of Diseases of,**  
Rosa, L. Wien. Klin. Wchnschr. 46: 1104, 1933.

Rosa describes the test below as of value in the diagnosis of hepatic diseases.

Set up 5 test tubes and introduce the following amounts of urine: 1, 2, 1.5, 2, and 5 c.c. Then add the following amounts of 10 per cent solution of silver nitrate: 0.2, 0.2, 0.1, 0.1, and 0.1 c.c. The resultant dilutions are thus 1:5, 1:10, 1:15, 1:20, and 1:50. While frequently shaking boil the tubes for from thirty to forty seconds in a water-bath. Remove from the bath and allow the precipitate to settle.

Negative reaction: sediment, snow white; supernatant fluid transparent yellow.

Positive reaction: lilac, brown, or black precipitate with supernatant fluid of same color.

The more severe the pathologic process, the higher the dilution in which the reaction occurs.

The reaction is related to the sodium chloride concentration of the urine.

**TUBERCULOSIS, Vernes Flocculation Test in,** Murray, R. South African M. J. 7: 645, 1933.

From a study of 121 cases Murray concludes that the sedimentation rate and the Vernes test do not appear to be related in any simple manner.

In definitely diagnosed cases of tuberculosis the Vernes test would not appear to be invariably positive, there being negative results in some 6.4 per cent of cases.

In cases where recovery is taking place, the sedimentation test appears to give a normal reading sooner than the Vernes test.

**MILK, Preservation of for Streptococcus and Abortus Examination,** Bryan, C. S. Am. J. Pub. Health 23: 1182, 1933.

A 1:10,000 concentration of brilliant green (0.1 c.c. of 1 per cent aqueous solution to 10 c.c. of milk) was found highly satisfactory.

**MALARIA, Vital Staining of Mitochondria in,** Boucher, H. Compt. rend. Soc. de biol. 112: 882, 1933.

Mitochondria can be demonstrated in the malaria parasite *Plasmodium vivax* by vital staining with Janus green.

The stain is prepared as follows: Of a saturated solution in absolute alcohol of neutral red 0.4 c.c. is added to 10 c.c. of absolute alcohol to which is added also three drops of a saturated alcoholic solution of Janus green. A drop of this is allowed to dry on a perfectly clean slide and the moist blood preparation is made on the thin film of stain. The mitochondria in the form of spherical granules stained by the Janus green half a micron in diameter vary in number from 1 to 20 according to the size of the parasite. The neutral red stains granules in the red blood corpuscles, the rose coloration assumed by the cytoplasm of the parasite being due to a reduction product of the Janus green.

**BLOOD CHOLESTEROL, In Chronic Nephritis and Retention Uremia, Ashe, B. I., and Bruger, M. Am. J. M. Sc. 186: 670, 1933.**

In cases of chronic Bright's disease with marked nitrogen retention, a low plasma cholesterol foretells death in uremia. In most cases this low reading is observed at an appreciable interval before death; in rare cases such a level is found only about twenty-four to forty-eight hours before exitus.

An elevated plasma cholesterol indicates a good prognosis as to recovery from pre-uremic symptoms but does not preclude the possibility of death from causes other than uremia.

There appears to be no close relation between the height of the plasma cholesterol and the degree of edema in patients with chronic Bright's disease.

There is some clinical evidence of a reciprocal relation between the blood urea and the plasma cholesterol; at other times this relation is offset by other factors. A constant inverse relation between the plasma proteins and cholesterol was not observed.

The diminished cholesterol content of the plasma observed in cases of Bright's disease dying of retention uremia is probably due to such contributing factors as cachexia and anemia.

**FIBRINOGEN, Quantitative Determination of in the Blood, Boehm, G. Diagn. E. Techn. di Lab. 4: 683, 1933.**

The author describes a simple technic as follows:

1. Fill and empty a sterile 10 c.c. glass syringe with 20 per cent potassium oxalate, thus leaving a thin film in the syringe.

2. Withdraw from 6 to 7 c.c. of blood by venipuncture, place in a test tube and centrifuge.

3. Remove 1 c.c. of plasma and dilute with 9 c.c. of 0.85 per cent saline and from this 1:10 dilution prepare a 1:100 dilution.

4. To a series of 16 tubes from 8 to 10 mm. in diameter add plasma and saline as shown in the table below:

| TUBE | PLASMA C.C.   | SALINE C.C. | CONCENTRATION OF PLASMA |
|------|---------------|-------------|-------------------------|
| 1    | 1.0 of 1: 10  | 0           | 1.0                     |
| 2    | 0.7 of 1: 10  | 0.3         | 0.07                    |
| 3    | 0.5 of 1: 10  | 0.5         | 0.05                    |
| 4    | 0.4 of 1: 10  | 0.6         | 0.04                    |
| 5    | 0.35 of 1: 10 | 0.65        | 0.035                   |
| 6    | 0.3 of 1: 10  | 0.7         | 0.03                    |
| 7    | 0.25 of 1: 10 | 0.75        | 0.025                   |
| 8    | 0.2 of 1: 10  | 0.8         | 0.2                     |
| 9    | 0.15 of 1: 10 | 0.85        | 0.015                   |
| 10   | 1.0 of 1:100  | 0           | 0.01                    |
| 11   | 0.9 of 1:100  | 0.1         | 0.009                   |
| 12   | 0.8 of 1:100  | 0.2         | 0.008                   |
| 13   | 0.7 of 1:100  | 0.3         | 0.007                   |
| 14   | 0.6 of 1:100  | 0.4         | 0.006                   |
| 15   | 0.55 of 1:100 | 0.45        | 0.0055                  |
| 16   | 0.5 of 1:100  | 0.5         | 0.005                   |

5. To each tube add 1 drop of 10 per cent calcium chloride solution and allow to stand in the ice box for thirty minutes.

6. Read for (a) the dilution showing a definite turbidity and (b) the dilution showing complete coagulation when the tube is inclined.

The amount of fibrinogen is determined by the formula:

(Unit) of fibrinogen. (U.Fg.) =  $\frac{1}{p}$  where p equals the amount of plasma in the tube (as shown in the table).

The author gives the normal as 40 or 60 U.Fg. per 1 c.c. of plasma.

**SYPHILIS, The Value of the Sachs-Witebsky Reaction (Citochol) for the Diagnosis of,**  
 Sebastion, F. *Diagn. E. Techn. di Lab.* 4: 641, 1933.

The author reports favorably upon this test which he believes to be specific, sensitive, and easy to read.

His technic follows: To 0.2 c.c. of serum inactivated at 55° C. for thirty minutes add 0.1 c.c. of 1:3 Citochol-saline mixture which has been allowed to ripen for thirty minutes at room temperature.

After twenty minutes add 1 c.c. of 0.85 per cent saline solution and shake. Readings are made after one hour, positive reactions being indicated by flocculation, the mixture remaining clear and limpid in a negative reaction.

**ANEMIA, Production of Erythrocytes, Reticulocytes, and Hemoglobin in,** Murphy, W. P.  
*Arch. Int. Med.* 52: 829, 1933.

It is well known that the magnitude, and to some extent the rate, of the response of the reticulocytes to therapy is in part dependent on the method of staining and technic of counting the reticulocytes, the number of erythrocytes present when treatment is started and the state of health of the patients in certain respects other than the anemia.

If the various secondary factors which influence the rise in reticulocytes are assumed to be constant, the magnitude of the response of the reticulocytes will indicate the potency of the material used if it is administered in submaximal amounts. The data presented, particularly for patients treated with maximal amounts of potent material, as with a concentrated solution of liver extract administered intramuscularly, indicate that a reticulocyte response of equal magnitude may be observed following the use of varying amounts of equally potent material, that the response will occur more quickly following the initial administration of large amounts, that the magnitude of the increase in erythrocytes may not vary directly with the magnitude and duration of the rise in reticulocytes and that the rate of increase of the erythrocytes is a more accurate indication of the effectiveness and potency of substances used in the treatment of patients with pernicious anemia than is the rate and magnitude of the reticulocyte response.

The explanation for the lack of correlation between increases in reticulocytes and erythrocytes as observed following treatment with various substances is not obvious and is probably not entirely controlled by the condition of the blood-forming organs at the time treatment is started. The variation in response to treatment by different means may be dependent on the rate of absorption or the utilization of the active principle under the various methods of its administration. If this is the case, it is probable that when administered in large amounts, as by intramuscular injection, the active material presented ready for the immediate use of the blood-forming organs causes more rapid maturation of the erythrocytes, so that the proportion of mature to immature cells (reticulocytes) in the circulation is greater than when less of the potent substance is available.

Is it possible, however, that two factors are present in the various materials used in the treatment for pernicious anemia, a stimulating (or reticulocyte-producing) and a maturing (or erythrocyte-producing) one? These may occur in varying proportions in different materials which have been used, the maturing occurring in greater proportion in the more refined and concentrated liver extract for intramuscular use.

In the treatment of the patient whose blood is in a state of relapse, it is desirable that improvement of this condition be promoted as rapidly as possible. Observations indicating an unusually rapid increase in erythrocytes following the intramuscular injection of initial large amounts of a concentrated solution of liver extract (12 c.c. prepared from 400 gm. of liver) and followed by infrequent smaller injections are presented. That this material is of a high degree of potency and that treatment by this means insures optimal efficacy are well illustrated by the improvement shown.

The administration of large doses of iron during treatment with liver extract parenterally is strongly recommended. By this means one may be more certain to obtain the maximum

effect of the liver material by preventing the development of deficiency in iron as a result of the rapid maturation of erythrocytes produced by the parenteral method of therapy.

**RHEUMATIC FEVER, Intravenous Vaccination With Streptococci, Wilson, M. G., Josephi, M. G., and Long, D. M.** Am. J. Dis. Child. 46: 1330, 1933.

The curves of the seasonal incidence of recurrence of rheumatic fever for subjects receiving hemolytic streptococcus vaccine therapy in 1928 and 1929 show a definite upward trend in 1931 as compared with the control group.

Twenty-three (19 per cent) of the treated subjects as compared with 15 (17 per cent) of the controls remained free from evidence of rheumatic activity for the three-year period of observation.

The annual incidence of recurrence for subjects receiving hemolytic streptococcus vaccine therapy was 48 per cent for 1929, 39 per cent for 1930 and 37 per cent for 1931.

The subjects who received a second course of treatment with hemolytic streptococcus vaccine in 1929 exhibited more recurrences in 1930 than subjects receiving only a single course of treatment.

The first series of 26 children who received typhoid vaccine intravenously in the fall of 1930 shows a diminished incidence of recurrence comparable to that observed following hemolytic streptococcus vaccine in the previous investigation.

The incidence of recurrence following streptococcus vaccine (polyvalent streptococcus B E and *Streptococcus viridans*, autogenous, individual and pooled) did not seem to be influenced by treatment.

There was no significant difference in the incidence of recurrence or manifestations of activity following intravenous therapy with various antigens.

The results secured in this investigation would seem to support the view that the temporary diminished incidence of recurrence observed to follow intravenous treatment with hemolytic streptococcus vaccine in the previous investigation was probably unrelated to therapy.

Intravenous vaccination with various antigens in the dosage used did not seem to influence the incidence of recurrence of rheumatic activity in the children studied.

**THYROID DYSFUNCTION, Gastric Acidity in, Wilkinson, S. A.** J. A. M. A. 101: 2097, 1933.

Of 100 cases of hyperthyroidism, 36 per cent showed achlorhydria, and the average acid of all cases was reduced to slightly more than half the normal.

The observation is made that the incidence of achlorhydria in hyperthyroidism rises in proportion to the duration of the toxicity rather than the degree of the toxicity.

After thyroidectomy, only 10.5 per cent show achlorhydria. The average free acid is raised to about the normal value for the entire series.

A theory is offered that the depression of gastric acidity is a phenomenon of extreme sympathetic overstimulation.

Hypothyroidism, in this series at least, produces a definite tendency to hyperacidity.

**ANEMIA, Chronic Idiopathic Hypochromic, Mettler, S. R., Kellogg, F., and Rinehart, J. F.** Am. J. M. Sc. 186: 694, 1933.

Data are presented of studies made on the relationship of diet and nutrition to anemia in 10 cases of chronic idiopathic hypochromic anemia associated with hypochlorhydria or achlorhydria and defective diet.

The response of the bone marrow, as determined by the hemoglobin and production of erythrocytes, to iron administered in large daily oral doses in the form of iron and ammonium citrate (U.S.P.) was compared to the response in patients on an "iron rich" diet. In addition, a comparison was made of the response of the bone marrow to a diet rich in iron and to a meal previously digested in vitro with hydrochloric acid and commercial pepsin.

The bone marrow responded rapidly and excellently to large doses of inorganic iron, but there was no evidence of increased hematopoiesis after the ingestion of an "iron rich" diet for a long period of time. There was, however, a rapid and satisfactory increase in concentration of hemoglobin, production of red cells, and a slight reticulocyte response following the administration of predigested meals.

It is concluded, from these studies, that chronic idiopathic hypochromic anemia is one presumably due to a deficiency of iron wherein gastric dysfunction leads to failure in utilization of organic (dietary) iron.

**EOSINOPHILIA, Induction of in Normal Animals, Banerji, N.** *Am. J. M. Sc.* 186: 639, 1933.

An elevation in the relative number of eosinophiles was induced in rabbits subjected to increased carbon dioxide tension of the blood, either by intravenous administration of carbon dioxide solutions or by exposure of the animal to an atmosphere containing increasing amounts of this gas.

From this result, it is concluded that an alteration in the acid base equilibrium of the body appears to incite an eosinophilia.

Further studies, it is hoped, will lead to a better understanding of the mechanism of this alteration.

**CANCER, The Reaction of the Arterial Blood in, Dickinson, S., and Havard, R. E.** *Brit. J. Exper. Path.* 14: 394, 1933.

The results of previous workers are summarized and discussed.

The technic is described of collection of samples of arterial blood from patients with and without malignant diseases, and the measurement of the  $P_H$  of the blood at 37° C., by two independent glass electrodes.

The results support the conclusion that the blood of patients with malignant disease of the mouth or skin, or with secondary glands, is not more alkaline than that of patients free from malignant disease.

An experiment is described that demonstrated that momentary alterations of respiration can affect the  $P_H$  of the blood.

**ENTERITIS, Polytropous (Acute Infectious Gastro-Enteritis, Spencer's Disease), Wildman, H. A.** *Arch. Int. Med.* 52: 959, 1933.

There apparently exists quite generally a clinical entity which has erroneously been called gastrointestinal influenza, food poisoning, ptomaine poisoning, biliousness, acute auto-intoxication, dietary indiscretion and the like. Considerable information concerning the etiology, epidemiology, pathogenesis, symptomatology and differential diagnosis of this condition has been gathered from observations on more than a thousand cases seen within the past seven and a half years. A distinctive name is now proposed. The present report is based on a study of about 750 college students who had from one to six or more attacks between September, 1927, and June, 1932. Much more work is needed to settle some points, particularly those concerning the etiologic agent and the pathologic condition which it produces.

**ACETANILID, Influence of Caffeine on Effects of, Higgins and McGuigan.** *J. Pharmacol. & Exper. Therap.* 48: 276, 1935.

Study upon animals of the effects of acetanilid plus caffeine did not present evidence of increased acetanilid toxicity. Mice receiving daily in their drinking water 50 per cent of the fatal hypodermic dose of acetanilid and 20 per cent of the fatal hypodermic dose of caffeine showed practically no effects from the drugs. The enormous dose with their food (0.5 per cent acetanilid and 0.5 per cent acetanilid plus 0.1 per cent caffeine or 650 mg. per

kilogram body weight) served only to delay growth in mice. Caffeine seemed only to lessen the toxicity. After six weeks the animals were placed on normal diet and rapid gain in weight followed.

Three hundred and twenty-five mg. acetanilid plus 62 mg. caffeine per kilogram per day had little or no effect on growth.

In rabbits the fatal dose of acetanilid is about 1.5 gm. per kilogram. In anesthetized dogs fatal doses cause respiration to stop before the heart. In therapeutic amounts caffeine would not add to the toxicity of acetanilid.

**SYPHILIS, Conjugal, A Statistical Study, Decker, H. B. Am. J. M. Sc. 187: 111, 1934.**

There is presented an investigation of the records of 376 families in which syphilis was present in at least one member of the conjugal union.

Sixty husbands and 98 wives were syphilitic with nonsyphilitic spouses.

In 218 families both husband and wife were syphilitic.

It is concluded that the type of syphilis depends upon the individual rather than upon any special strain of treponema.

Late syphilis is relatively noninfectious. Infectivity varies inversely with the duration of the disease.

**GRANULOMA INGUINALE, Further Studies on the Etiology of, De Monbreun, W. A., and Goodpasture, E. W. Am. J. Trop. Med. 13: 447, 1933.**

A gram-negative bacillus belonging to the aerogenes group has been isolated from the lesions of two cases of granuloma inguinale, and from the feces of two out of four other cases of the disease.

This bacillus reproduces exactly the morphological characteristics of the Donovan microorganism, including the typical Donovan body which appears as a secondary phenomenon in older cultures in blood media.

Cultures of this bacillus have failed to reproduce the specific lesions of granuloma inguinale when inoculated into lower animals and into man.

The Donovan microorganism apparently increases in mononuclear cells in tissue cultures in the primary explants from human lesions, but has failed to grow in subcultures.

Notwithstanding failure to reproduce granuloma inguinale experimentally with cultures of the bacillus described, the hypothesis that this disease is primarily a chronic intestinal infection with a member of the aerogenes group of bacilli, with secondary infection of the skin from constant fecal contamination is advanced as worthy of further investigation.

**ACTINOMYCETES, Modified Sabouraud Medium for Cultivation of Acid-Fast, Scudder, S. A. Science 79: 16, 1934.**

The following medium was found satisfactory:

Maltose, 4 per cent; Difco peptone, 1 per cent; flaked agar, 1.8 per cent; dissolved in unfiltered beef heart or veal infusion instead of water. No adjustment of reaction is necessary. If desired, glycerin and other carbohydrates may be added. The use of the medium as slants favors the development of acid-fastness in about four days. Simultaneously with the appearance of the acid-fast portions of the growth, a grayish-brown powdery substance develops upon the upper portion of the slant.

**MICROSCOPY, Using a "Dry" Microscope Objective on Uncovered Objects, Sauer, F. C. Science 78: 537, 1933.**

By attaching a cover glass temporarily to the lens (by a film of cedar oil, mineral oil, or water) the study of uncovered objects is greatly improved.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### Der Krebs, seine Entstehung und Erklärung\*

A THEORETICAL discussion which defines cancer as a biologic result of the disturbance of the three factors which regulate cell life, that is (1) a disturbance of the life forces resident in the cell, (2) a lack of normal hormonal influence or excess of pathological hormonal influence, and (3) lack of the trophic innervation of the vegetative nervous system. Disturbance of all three factors causes the revolutionary cancerous transformation of the cell; disturbance of two factors produces the precancerous stage.

### Die normale und pathologische Physiologie der Milz†

THE author modestly acknowledges the scanty and conflicting evidence concerning the functions of the spleen and states that he has spent years in personal attempts to decide certain disputed points. He hopes that his book may be of assistance to others working in this dark and difficult field. One of the anomalies is the fact that removal of the spleen in man causes no detectable symptoms while its removal in rats and many other experimental animals results in a few days in a Bartonella infection and a rapidly fatal anemia. Apparently most laboratory animals harbor a latent Bartonella infection which is normally held in check by the spleen but which runs wild after splenectomy. In the various chapters the spleen is considered as a blood reservoir, as a hematopoietic and hemolytic organ, as a protection against infections and tumors, as it influences the digestive tract and metabolism, and as an organ of internal secretion.

### Das Chlorophyll als Pharmakon‡

CHLOROPHYLL, the common green respiratory pigment of the plant world, has recently been studied intensively by Willstätter and others, and it has been found to be closely related in chemical structure to hemoglobin, the common red respiratory pigment of the animal world. The nucleus of each consists of a ring structure made up of four pyrrole groups and a metal, iron in hemoglobin and magnesium in chlorophyll. In cases of anemia it would seem a natural and logical indication to supply this pyrrole nucleus for the building of new blood. In fact, both blood and chlorophyll have been so used but the results are no better than are obtained by the administration of simple forms of iron. Bürgi, however, finds that chlorophyll has a very general tonic effect on all the organs and functions of the body; it tones the heart and blood vessels, it increases peristalsis, it lessens the fatigue of nerve muscle preparations, etc. He finds that rabbits fed green vegetables are brighter and more active, and we all know that the old cow is rejuvenated when she gets green grass in the spring. Green vegetables, however, contain also carotinoids, vitamins and other products, but Bürgi finds that extracted chlorophyll has this same tonic effect and is more completely absorbed than is chlorophyll taken in the form of green vegetables.

\*Der Krebs, seine Entstehung und Erklärung: eine biologische Studie. (Cancer, Its Origin and Explanation, a Biological Study.) By A. W. Kukowka. Wilhelm Maudrich, Vienna, 1932.

†Die normale und pathologische Physiologie der Milz. (The Normal and Pathological Physiology of the Spleen.) By Ernst Lauth. Urban & Schwarzenberg, Berlin, 1933.

‡Das Chlorophyll als Pharmakon. By Emil Bürgi. Georg Thieme, Leipzig, 1932.

### The Anatomy of the Rhesus Monkey\*

CONSIDERING the extensive use of the rhesus monkey as a laboratory animal, it is strange that some such publication as this volume has not hitherto been available.

While not, strictly speaking, a textbook, the volume nevertheless represents the first comprehensive description of the anatomy of the old world monkey and as such may justly be regarded as a pioneer in this field.

It consists of eighteen chapters representing the studies of competent contributors thoroughly familiar with their subject.

Each contributes a detailed study of a definite field so that the volume as a whole covers the subject in its entirety.

It is, of course, not to be expected that the volume should approximate in character the textbooks of human anatomy because of the limited numbers of dissections and anatomical investigations upon which its studies are based.

Nevertheless, the research worker and the student of comparative anatomy will find in its pages much that has not before been subjected to extensive study or comprehensive description and while future studies may, to some extent, lead to modification or revision of this or that section or portion of a section, the work represents a contribution of great value and definite utility.

Very wisely, it seems to the reviewer, and contributing to the usefulness of the book, the B. N. A. nomenclature, rather than that of comparative anatomy, has been used throughout.

An appendix contains an excellently written and illustrated account of the housing and care of monkeys which should prove most useful.

The contributors, editors, and publishers may be congratulated upon the production of a useful volume.

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### A System of Clinical Medicine†

THE fact that this book, addressed particularly to students and practitioners, has reached a ninth edition is *prima facie* evidence of its practical character and usefulness which becomes evident even with the most cursory perusal of its pages.

There are many books concerned with clinical medicine and the problems confronting the practitioner, all having their place and value. Few, however, will in a broad sense possess any greater utility than Savill's.

This is largely due to the somewhat different, and original plan upon which it is based in presenting the subject from the purely clinical standpoint, the bedside point of view, as it were, in which the problem confronting the physician is to correlate the symptoms observed with their underlying cause.

The discussion of each condition, therefore, is approached from the following angles:

First, a discussion of the symptoms and their possible causes in which is presented a comprehensive outline of the signs indicative of disease in the organ or region under discussion, as well as a discussion of the fallacies incident to their detection and a differential account of their various possible causes.

Second, a discussion of the physical signs in that region and the methods of eliciting them.

Third, a clinical classification of the various diseases affecting the region in question and a discussion of the various diseases arranged in accordance with their clinical relationships.

By this arrangement the reader is presented with a very useful clinical index of diseases and finds himself reading the diagnosis, prognosis, and treatment of the disease at

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†A System of Clinical Medicine. By Thomas Dixon Savill, M.D. Ninth edition, cloth, pp. 1063, 163 illustrations, 6 colored plates. William Wood and Co., Baltimore, Md.



hand and also, in immediate proximity finds a description of conditions which clinically and pathologically often more or less resemble it and thus may be mistaken for it.

For any degree of success the carrying out of such a plan necessarily requires a foundation of broad and digested experience. That Dr. Savill has been successful in his project the volume itself bears witness.

The present edition, thoroughly revised and brought up to date, brings to the practitioner and student an exceedingly useful and well-written presentation.

### Food-Borne Infections and Intoxications\*

WHILE food poisonings and food infections are relatively unimportant as a cause of death, a sufficient number of outbreaks occur to warrant an understanding of their nature and mechanism.

In this book, a companion volume to the author's previous text upon *The Microbiology of Foods*, food infections and food intoxications are presented in a thoroughly comprehensive and satisfactory manner.

Those interested in this subject will find in this book practically the information available concerning every type of food infection or poisoning on record. Under each heading will be found illustrative case reports and records of outbreaks, the bibliography being exceptionally full and complete. The style is clear, simple, and eminently understandable.

This book and its companion should be of interest, not only to the physician who may be confronted with outbreaks of this character as well as with others simulating food poisoning but really something else, but especially to health officers and laboratory workers who may be called upon to study such outbreaks.

It can be recommended as a thorough and comprehensive study of a subject not always clearly understood.

### Textbook of Pathology†

THIS well-known book requires no introduction, having served as a standard text for the last decade.

In this, the third edition, will be found a number of changes and additions, not only in the text, some portions of which have been entirely rewritten, but also as concerns the illustrations, a number of which are new and all of which in general will be found adequate.

While following in general the common division of the subject into General and Special Pathology, this distinction has not been overemphasized. Based upon the author's experience as a pathologist and teacher, the volume will be found practical and of value, not only to the students to whom it is particularly addressed, but also to the clinician and, as heretofore, as a work of reference.

There is an adequate index.

### Die Spezifität der serologischen Reaktionen‡

THIS is an attempt to bring together, coordinate, and interpret for German readers the results of his own investigations and those of his coworkers and to discuss the very difficult problems of biological specificity, especially from the chemical standpoint. He takes up in order the specificity of proteins, of cell antigens and antibodies; then the specificity of serological reactions with artificial complex antigens and finally the specific cellular substances such as the specific carbohydrates and lipids. It has copious references and an index.

\*Food-Borne Infections and Intoxications. By Fred Wilbur Tanner, Ph.D., Professor of Bacteriology, University of Illinois. Cloth, pp. 429. The Twin City Printing Co., Champaign, Ill.

†Textbook of Pathology. By Robert Muir, Professor of Pathology, University of Glasgow. Third edition, cloth, pp. 957, 548 illustrations. William Wood and Co., Baltimore, Md.

‡Die Spezifität der serologischen Reaktionen. By K. Landsteiner. Julius Springer, Berlin, 1937.

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The rate of disabling illness in families of the unemployed was 25 per cent higher than that in families containing part-time workers and 39 per cent higher than that among families of full-time wage earners.

These conclusions are such as one would anticipate, and could easily form the basis for generalizations regarding the effect of a financial depression on sickness in general. The authors, however, very wisely emphasize that conclusions should be drawn only on the material studied. They make no attempt to draw broad implications and caution the reader to exercise similar restraint. The group is relatively small and does not necessarily represent a cross-section for the United States. They also point out that specific interpretation of increased illness rate in terms of impaired vitality cannot be made without knowledge of the nature of the illnesses experienced.

In this study the results were what one would expect. In another recent analysis<sup>2</sup> they were the reverse. Deaths from tuberculosis in 28 states surveyed were lower in 1932 than in any of the preceding four years. The fall in rate has followed the regular established curve for tuberculosis, apparently uninfluenced by the depression. The rate per 100,000 in 1928 was 77.3; in 1932, 60.4.

A very interesting example of the difficulty of drawing statistical conclusions from relatively small figures is reported by C. M. Smith in his study of housing conditions and respiratory disease in Glasgow.<sup>3</sup> This also was in a measure an economic survey. The prevalence of respiratory disease was studied in two Glasgow areas, one a poor-class quarter, a slum district, and the other a rehousing area. The latter corresponds to our model tenement developments. The survey covered an entire year. The inhabitants of the two areas, numbering about 1,000 each, were in comparable economic states, but the housing facilities in one group were presumably much more sanitary than in the other.

To our surprise we find that there was a greater morbidity from acute respiratory disorders in the rehousing area than in the slum quarter. Here we are presented with an apparent paradox to the anticipated, and the explanations presented are based upon isolated observations rather than figures. Smith states that the results cannot be taken as evidence that improved housing conditions have been without effect on the prevalence of respiratory disease for the following reasons. In the slum district 20 per cent of the population were foreigners, chiefly Jews and Lithuanians, among whom there was less unemployment and dietary habits were better than in the rest of the population. The willingness of informants to report cases is a factor, and the author believes that reporting of illness was more accurate in the model tenement group. Theoretically, provision in the model tenement district allowed for fewer persons per room, there being only about one-fifth as much overcrowding. As a matter of fact a large proportion of the rehoused population, not long removed from the slums, continued to use just one of their several rooms for sleeping, especially in cold weather. Therefore, during the night, crowding was just as prevalent

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Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIAL

### The Hazards of Statistical Interpretation

A SURVEY of the effect of the economic depression upon sickness has been undertaken by Perrott, Collins and Sydenstricker.<sup>1</sup> The completed analysis will be based upon the records of illness in 1933 as compared with the economic history from 1929 to 1932, in more than 12,000 families in eight large cities, a group of coal mining communities, and a group of cotton mill villages. The authors' preliminary report presents the preliminary results in three of the large cities, Birmingham, Detroit, and Pittsburgh. This comprises a group of 2,566 families, 11,330 individuals. They find a definitely higher incidence of disabling illness among individuals in the lower income classes than among those with higher incomes. The highest sickness rate is reported in the group which in 1929 had been in reasonably comfortable circumstances but which in 1932 had dropped to relative poverty. The sickness rate in this group is 60 per cent higher than among those who in 1929 were in comfortable economic

state and who maintained that state through 1932. The rate was even higher than that for the "chronically poor" group, those who were poverty stricken in 1929 and 1932.

The rate of disabling illness in families of the unemployed was 25 per cent higher than that in families containing part-time workers and 39 per cent higher than that among families of full-time wage earners.

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in the rehousing district. The study illustrates beautifully some of the difficulties encountered in undertaking field surveys and drawing conclusions therefrom.

There is perhaps a natural tendency in making any statistical analysis, however small, to accept as accurate those figures which appear to substantiate what one's judgment or one's theory leads one to believe to be correct. Too often one is inclined to overlook the tremendous number of units required before all variables become negligible. The requisite total number varies, naturally, with the subject matter studied. Also, unfortunately, in many types of surveys in which statistics must be employed, sufficiently large numbers are actually not available.

This should not rule out the possibility of recourse to statistical methods, but when these are used one would do well in drawing conclusions, even though they appear entirely correct, to adopt the cautiousness manifested by Perrott, Collins and Sydenstricker, in applying conclusions only to the material studied and avoiding unjustified generalizations.

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2. Public Health Reports 48: 479, 1933.
3. Smith, C. M.: *Housing Conditions and Respiratory Disease. Morbidity in a Poor-Class Quarter and in a Rehousing Area in Glasgow*, Medical Research Council Special Report Series, No. 192 (London), 1934.

—W. T. V.

# The Journal of Laboratory and Clinical Medicine

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## CLINICAL AND EXPERIMENTAL

### INTRACELLULAR STRUCTURES IN MONOCYTES IN CASES OF MALIGNANT DISEASE\*

O. C. GRUSER, M.D., MONTREAL, CANADA

THE occurrence of bodies of spiral form within the large mononuclear leucocytes in cases of malignant disease was noted in 1916<sup>1</sup> but without arriving at any definite conclusion as to their nature. It was considered possible that their presence might afford a means of diagnosis.

Since that time, a search for bodies of this kind has been made in every subsequent available case, whether hospital or private (in England), and it was found that when they were present, the case was very often one of malignant disease. It was, therefore, concluded that their presence could not be accidental, or a coincidence, but that they must represent genuine structures and have a positive significance. The problem remained as to whether they were possibly mitochondrial in nature, or perhaps belonged to the category of the inclusion bodies described in the case of virus diseases by various observers.

*Technical Note.*—Ordinary air-dried blood films are used. They are stained either by Pappenheim's panoptic method or by Leishman's stain. The latter is time saving and satisfactory. No other methods of staining fulfill the requirements. A strong light is essential for the examination. The endothelioid monocytes are specially studied. When the filamentous forms are not very distinct they are traceable by searching first for their nodal points.

*Forms Noted.*—Coccoid and bacillary (batonnet) forms are well known to occur in the mononuclear leucocytes of normal blood and of exudates. They

\*From the Department of Surgery, McGill University.

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vary in size from the ordinary azure granule to the granules and rods known as chondriomes and mitochondria found in various tissue cells. It is not these that form the subject of this communication, but linear forms; namely, (a) irregularly wavy or filamentous lines which may assume a definite spirillar form. These correspond to the chondriocentes of cytology (Fig. 1). (b) Curved or angular lines marked by nodal thickenings. Some of these are definitely flexed at the nodal thickenings and may be called "geniculate bodies." (c) Branching lines or "mycelioid" bodies. There is sometimes a round thickening at one end as shown in Cell 6 of the figure. (d) Other forms include (1) "beaded" forms, which appear as rows of fine dots lying in a linear vacuole. These correspond to



Fig. 1.—Nine cells are shown, each from a different case of malignant disease. Eight show cytoplasmic structures, and one shows an intranuclear body. Magnification: Slightly larger than that apparent with the ordinary oil immersion lens and medium high ocular. Cells 1 and 2 are hyaline leucocytes. The rest are monocytes. *Coccal forms*: Cells 1, 2, 4, 5, and 7 show the ordinary fine azure granules. Cell 1 also shows a single granule in a vacuole and a diploid body in a vacuole. *Undulant and spiral forms* are shown in cells 3, 4, 5, and 7. *Noded or beaded lines* in cells 1, 2, 4, and 5. *Spirillar form* with globular end in cell 2. *Mycelioid form* in cell 6. *Protozoal form* in cell 9. Cell 7 was considered possibly a sarcoma cell.

chondriomites of general cytology, (2) intranuclear bodies in vacuoles, showing a very fine granulation (Cell 8 of figure), (3) larger bodies occasionally occur which might easily be mistaken for protozoa.

*Possible Errors of Interpretation.*—Spiral bodies may be simulated by edges of cell substance wrinkled by spreading the film, so that an optical effect is produced. Nodal points may be passed by as being simply azure granules, the filaments being overlooked. Azure granules may appear to be connected simply



through an optical effect. Granules in intracytoplasmic clefts may appear as chondriomites. Only forms which appear definitely red or purple red are accepted as genuine intracellular structures.

TABLE I

FREQUENCY WITH WHICH INTRACELLULAR BODIES WERE NOTED IN 100 MONONUCLEAR LEUCOCYTES IN POSITIVE CLASSES OF MALIGNANT DISEASE

(1925-1926)

| SITE<br>(TYPE)                   | NO.<br>CASES<br>EX-<br>AMINED | PER-<br>CENT | NUMBER OF BODIES OBSERVED |       |       |       | TOTAL |
|----------------------------------|-------------------------------|--------------|---------------------------|-------|-------|-------|-------|
|                                  |                               |              | 0-10                      | 10-20 | 20-30 | 30-40 |       |
| Alimentary tract                 | 77                            | 100          | 84                        | 20    | 6     | 22    | 122   |
| Epithelioma                      | 41                            | 92           | 71                        | 17    | 5     | 15    | 110   |
| Cervix and uterus                | 25                            | 90           | 61                        | 20    | 9     | 10    | 98    |
| Breast                           | 20                            | 100          | 35                        | 26    | 7     | 28    | 120   |
| Other sites                      | 27                            | 90           | 76                        | 19    | 9     | 11    | 113   |
| Sarcoma                          | 22                            | 87           | 50                        | 22    | 10    | 11    | 120   |
| Total untreated patients         | 223                           | 93           | 71                        | 21    | 7     | 7     | 91    |
| Patients under radium treatment  | 21                            | 73           | 51                        | 24    | 9     | 7     | 8     |
| Patients definitely nonmalignant | 435                           | 67           | 56                        | 62    | 604   | 21    | 8     |

*Comment.*—The third column shows that intracellular structures of the types described occur in a high percentage of cases of carcinoma or sarcoma. Cases are counted as negative if these bodies are not seen within the conditions selected, namely during the scrutiny of between 100 and 200 leucocytes. The last column indicates how soon the bodies will be seen. Thus, in the alimentary cases, 132 out of every 1,000 mononuclear leucocytes showed them; in other words, about every eighth mononuclear.

This serves to show that this form of study can come within the scope of routine work.

As regards the first column, cases in the alimentary tract include all tumors primary in the stomach or onward, that is, not epitheliomas. The term "epithelioma" covers cases which were external or buccal or in the upper alimentary tract as far as the cardiac orifice. The negative cases in this group were mostly esophageal. Sarcomas appear to provide most of the negative results, one case was a myxosarcoma and one a fibrosarcoma; there was one case of fibromyoma turning malignant. Perhaps it is not surprising that these should yield negative results.

The cases described as "definitely nonmalignant" fall into four groups: (1) definitely healthy persons, (2) students who were apparently in good health, (3) patients admitted to hospital without organic disease as far as known (hernia, fracture, hemorrhoids), (4) various medical and surgical cases considered to be certainly nonmalignant. Some of these gave positive results. Of these, details were available in six, two being cases of suppuration, one of tuberculosis with pyopneumothorax, one of simple goiter, one of Hodgkin's disease and one a chronic mastitis (therefore possibly precancerous).

A considerable number of "suspect" cases could not be included in the table, as it was felt that pathologic confirmation was desirable before the case was eligible, considering how effectively other diseases sometimes mimic cancer.

*Patients under Radium Treatment.*—A definite fall in the proportion of patients who show these bodies is noticed, and in the others a longer search was required before they could be discovered.

No note is here made of the relation between positive findings and *stage of disease*. It has been found that even histologically, early cases may show the inclusions. But some of the "negative" results were furnished by clinically early cases.

#### DISCUSSION

Filamentous and other cytoplasmic structures are considered by cytologists to be integral components of any cell. But they are not specially referred to in textbooks on hematology as being discernible in ordinary blood films. The exact counterparts of those under discussion are, however, figured in, for instance, E. B. Wilson's classical work on *The Cell*. Similar bodies are described and discussed in the literature on virus diseases, and the question as to their nature and significance may be discussed on the same lines.

A. They may be intrinsic in the cell: (i) normal products of cell metabolism, or of growth, or of cell division. Here belong mitochondria, filamentous chondriocentes, beaded chondriosomes, lepidosomes, metachromatic, secretory, or other granules, Golgi apparatus;<sup>9</sup> (ii) nuclear in origin; chromidia, spirem threads, chromosomes; (iii) degenerative products.

One has to decide between (i), (ii), and (iii) before deciding that a given cell really contains the particular bodies in question. Granules are not noted. Hence, the difficulty of deciding whether a granule is a degenerative product or a secretory product is evaded. The position of *filamentous mitochondria* in the cell economy is also not agreed upon by cytologists. For the present purpose, it is, however, convenient to refer to these bodies as mitochondrial, though they do not give the classical staining reactions.

Definite mitochondria can be seen quite frequently in neutrophile leucocytes, especially in pyogenic infections, as well as in malignant disease, presumably owing to the depletion of the granules, which renders them visible. But the similar bodies in the monocytes do not give the same staining reaction with Leishman stain.

They may be classified as chondriosomes in that the latter are regarded as phospholipoid in nature<sup>9</sup> (pp. 47 and 223), and Giemsa will stain such material the same way as it does these bodies.

*ii. Nuclear origin:* Chromidial extrusions are commonly seen in circulating blood cells, and they have been noted especially in cases of malignant disease. They are also numerous in health under a purin rich diet.<sup>3</sup> But these extrusions never attain the length of the bodies in question; they are of drumstick or spiculated form. Perhaps the occurrence of long ex-nuclear lines likewise denotes increased nuclear activity, material passing out into the cytoplasm.

The noded lines may be of nuclear origin also, if one regards the cytoplasm as permeated by canaliculi down which granules of nuclear matter proceed outward into the circumcellular fluid. The internodal lines would then be taken as optical effects produced by the canaliculi. The mycelioid forms might be

explained in a similar manner, the extranuclear material running into the canalicular network without separating into droplets or granules.

Such an explanation would justify taking the presence of these bodies in general as an *index of chromoplasmic activity*, and this would be in harmony with the generally favored view as to the nature of malignant disease.

The noded lines bear a resemblance to kinn threads. But this is likely to be accidental, for there is no reason why they should enter the cytoplasmic canals, especially considering that circulating leucocytes do not undergo mitosis. Portions of chromatin have, therefore, no opportunity to become detached or to fail to reenter daughter nuclei. In any event iron hematoxylin has not shown their presence.

B. They may be extrinsic to the cell. (i) Nonspecific to malignant disease: (a) artefacts, such as contamination with extraneous objects, pseudoparasitoses, physical staining effects; (b) phagocytosed organisms. (ii) Specific to the disease (a) in etiologic relationship, (b) not so.

Re (i) (b). In favor: (1) The Staining reaction. On the one hand, ordinary pathogenic bacteria do not stain red with Leishman stain, whether free or within neutrophile leucocytes. On the other, experiments made by me have shown that protozoa and mycomycetes from insect intestine stain the same color as to these mitochondrial bodies after phagocytosis by human leucocytes, using opsonic technic. In the case of the leucocytes in malignant disease both neutrophiles and monocytes may show the bodies, yet those in the neutrophiles stain like ordinary bacteria, whereas the others take on the red color. It is, therefore, feasible that one is dealing with phagocytosis in each case, but the type of organism is different or its reaction in the cell is different. (2) Carcinomatous tissue frequently contains bacteria. Ulcerated tumors are necessarily infected. But even other tumors prove to contain various organisms. It would appear that neoplastic tissue attracts various organisms which may be circulating (as they do even in health), so that they gradually accumulate in the tissue.<sup>8</sup> A number of recent publications discuss the bacterial content of tumors and suggest it as a cause of increased virulence of the neoplasm, especially in the cervix cases.<sup>1, 7</sup> In fact, bacteria might even be necessary to the growth of the neoplasm, so that the therapeutic attack should commence upon them.

Now the flora of these infected tumors is multiple. They, therefore, may provide both types, those taken up by the neutrophiles, and those by the macrophages, with the corresponding difference in staining effect. (3) Histologic study shows that there is at least every opportunity for both orders of cells (invariably present in and among the tumor cells) to take up these organisms and return to the blood. Fragments of mycelium, and noded or geniculate bodies have been identified in specimens from cases in which the blood has shown the inclusions.

Against: (1) even though ingested by monocytes, even the higher bacteria should be stainable with special bacterial stains, yet they are not. (2) There must be enormous numbers of these organisms circulating in the cancer case, seeing that some are present in every cubic millimeter.

Re ii. (Are they specific?) (a) In etiologic relationship? In favor: (1)

*Patients under Radium Treatment.*—A definite fall in the proportion of patients who show these bodies is noticed, and in the others a longer search was required before they could be discovered.

No note is here made of the relation between positive findings and *stage of disease*. It has been found that even histologically, early cases may show the inclusions. But some of the "negative" results were furnished by clinically early cases.

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They are found in the tumor cells also; (2) some authorities on virus diseases incline to the idea that the inclusions in those diseases are specific; (3) absence of ordinary staining reaction for known bacteria (at one time the mycelium of actinomycosis could not be demonstrated in the tissue-lesion); (4) the nodes in the geniculate bodies may be similar in function to the arthrospores of streptococci, etc., or of streptothrix.

Against: (1) The evidence that they may be simply phagocyted organisms which certainly do not cause cancer; (2) tumor cells are able to phagocyte such organisms (but the presence of such bodies is taken as itself evidence that tumor cells can ingest organisms); (3) most workers do not regard inclusion bodies in virus diseases as causal (on the other hand, this is merely an expression of opinion, either way); (4) they have not the individuality, e.g., of malarial plasmodia.

(b) Not in etiologic relationship? That is, are these bodies not the actual virus, but vectors of virus? In favor: (1) There is nothing intrinsically impossible in ex-nuclear material being a bearer of ferment or zymogen, seeing that intracellular ferments must inhere in some concrete cell constituent anyway. Therefore, such material can equally harbor a virus. (2) There is the analogy of the chromosomes, which bear highly complex potentialities.

Whatever be the origin and nature of these bodies, there remains one practical fact, namely, that the subject of malignant disease has his blood "loaded" with the material in question, even at an early stage in the case. Therefore, if the answer to question B ii (a) is positive, it would mean that the monocytes carry virus about the body, and that metastases might arise even if tumor cells did not become detached. The whole body would be diseased almost from the outset, and it would not be a matter simply of an initial local lesion. But if the answer to question A ii be positive, the findings in the blood films would be interpreted in the sense of a nonspecific chromoplastic activity, and one would understand why such conditions as extensive abscess formation sometimes also furnish similar bodies in the blood cells.

On that last view, the findings may simply reflect the outpouring of cytotoxic and cytotoxic products by the neoplasm itself, an event which need not be continuous, and need not occur in all stages. The new tissue may be "closed" by fibrosis, or "open" (only a lymphocyte barrier). In the former case the mononuclear leucocytes reveal nothing, whilst in the other case they contain these bodies. It has been found in the course of this study that the blood is actually not always equally rich in the mitochondrial bodies even at all times in the day. The nucleo-irritative products seem, therefore, to vary in amount, apart from the defensive tissue reaction. Perhaps this defensive reaction explains the disappearance of the inclusions after successful radium therapy.

It may be noted that in a few cases the benign or malignant nature of a palpable tumor could be decided correctly by means of this search for cell inclusions.

#### SUMMARY

A description is given of certain filamentous, spirillar, and geniculate structures seen in (especially) the monocytes in a high percentage of cases of malignant disease, and in very few cases which were clinically nonmalignant.

They are demonstrable only with Giemsa staining or by Leishman's stain.  
They may disappear from the blood after successful radium therapy.  
Their possible nature is discussed.

During the past year, liberal facilities for bringing this study to maturity were furnished by Dr. E. W. Archibald, Director of the Department of Surgery of McGill University, so that material became accessible from the Royal Victoria Hospital, the Montreal General Hospital, and other hospitals in Montreal. Thankful acknowledgment is here extended to the physicians and surgeons who placed their cases at disposal.

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## THE TOXEMIAS OF PREGNANCY\*

### II. THE NITROGEN METABOLISM

ALLAN WINTER ROWE, PH.D., MARY A. McMANUS, A.B., AND GERTRUDE A. RILEY, A.B., BOSTON, MASS.

IN A previous communication<sup>1</sup> the senior author has discussed some evidences of the hepatic factor in the group of widely diverse conditions generally called the "toxemias of pregnancy." These evidences were among the results of an elaborate chemical and laboratory study of fifty pregnant women who exhibited some one of these toxic conditions.

The basis of selection, the method of study, and the general composition of the group have already been considered in the paper noted above.

Data derived from the study of seventy-seven women studied repeatedly both throughout a normal pregnancy and for a number of weeks thereafter,<sup>2</sup> have served as a control; these will be drawn upon, as needed, for the present discussion.

This present paper is concerned with the protein metabolism, as evidenced chiefly in the levels of blood and urine nitrogen.

\*From the Evans Memorial, Massachusetts Memorial Hospitals.  
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<sup>2</sup>Read before the Section on Pathology and Physiology at the Eighty-Fourth Annual Session of the American Medical Association, Milwaukee, June 16, 1933.

The normal group was in part drawn from institutions for unmarried mothers, who were usually young primiparas. The toxemia group were older women, only one-third of whom were in their first pregnancy and not a few of whom gave histories of extensive childbearing. No evidence was forthcoming in the study that repeated fetation led to results differing from those characterizing the primiparas.

The time of study of the individual members of the toxic group was varied. Three-fourths of the patients fell in the last three months; the remainder showed only a general distribution.

In considering the protein exchange, the total nitrogen in the urine is

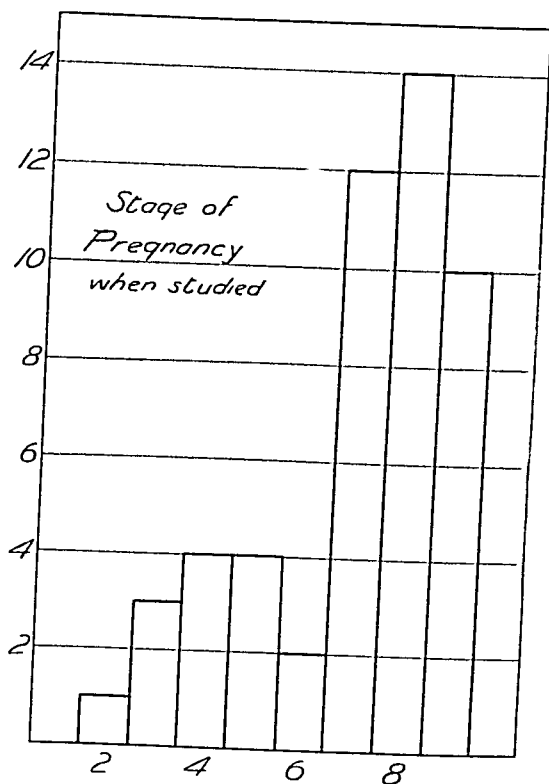


Chart 1.

significant as a measure of the absolute protein utilization, since skin elimination under most circumstances is but an inconsiderable fraction. Further, the independent quantitation of several individual components whose summation determines the total value is significant, as the metabolic origins of each fraction are widely differentiated. The original metabolized protein is the sole factor common to them all. To illustrate, urea is the indifferent form in which the principal amino-acid waste is eliminated, while ammonia, on the other hand, is the end-result of a defense mechanism mediated by the kidney to aid in the elimination of certain acid metabolites without depleting the blood of its essential fixed alkali. The so-called "nitrogen partition" deals with the four major components, urea, uric acid, ammonia, and cre-



atinine, and then assembles the remainder of the numerous nitrogen-containing substances into a single fraction called the "residual" nitrogen, which is determined by difference. Folin<sup>1</sup> established certain criteria for normal partition values and the few later studies on normals (Matthews,<sup>1a</sup> Long and Gephart,<sup>1b</sup> Rowe and Proctor<sup>1c</sup>) have been broadly confirmatory. The last authors<sup>1c</sup> confirmed earlier observations that the residual fraction showed a relative increase when the total nitrogen was diminished and by a synthesis from their own data, with those of Folin (l.c.) and Smith,<sup>2</sup> defined the curve representing this relationship.

Briefly, with a total elimination exceeding 8 gm., there is seemingly a fixed relationship at something less than 6 per cent. At levels slightly below this minimum the curve takes a sharp progressive upward trend and soon assumes large magnitudes. In a number of studies the senior author has defined the upper normal level of residual nitrogen at 9 per cent where the total elimination indicates a protein exchange above the conventional main-

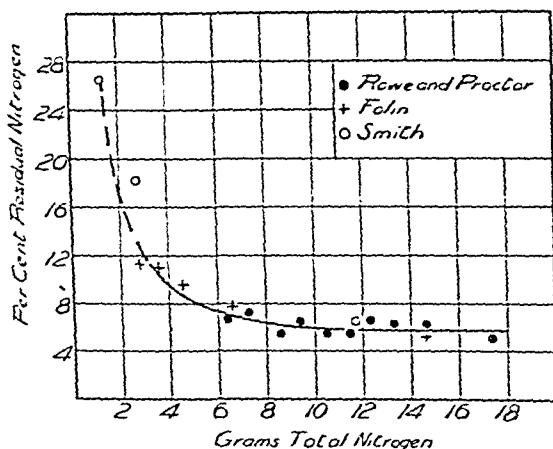


Chart 2.

tenance level of 7 gm. of  $N_2$ . Further, he has shown that values above this rather generous limit are one general index of a disturbed metabolism arising from a very wide variety of causes. This value then assumes a special significance in the assessment of the normality of the individual metabolism. In an earlier paper<sup>6</sup> dealing with the normal control group, the average nitrogen elimination was reported as relatively constant, at least during the last twenty-four weeks of pregnancy, and lying at a level slightly superior to maintenance. When one remembers, however, that the mother is storing protein in amounts far in excess of fetal needs, the adequacy of the catabolic exchange shown by the urine level assumes an added significance.

Turning now to the actual results of the study, the significant figures are grouped in tabular form (Table I).

The identity of the two total nitrogen values in the normal series ante- and postpartum, is fortuitous. Comment should be made, however, on the fact that following delivery the antepartum storage seems to be replaced by a different factor which also produces the common end-result of low urine

TABLE I  
NITROGEN PARTITION IN URINE

| DATUM                               | NORMAL |      | TOXIC |      |
|-------------------------------------|--------|------|-------|------|
|                                     | A.P.   | P.P. | A.P.  | P.P. |
| Total nitrogen (gm.)                | 8.03   | 8.04 | 6.93  | 8.33 |
| Urea nitrogen (%)                   | 79.6   | 80.8 | 77.8  | 80.8 |
| Uric acid nitrogen (%)              | 2.8    | 1.9  | 3.1   | 2.8  |
| Ammonia nitrogen (%)                | 5.0    | 5.1  | 4.2   | 3.3  |
| Creatinine nitrogen (%)             | 4.7    | 4.2  | 4.7   | 3.7  |
| Residual nitrogen (%)               | 7.9    | 8.0  | 10.2  | 9.4  |
| Per cent with residual more than 9% | 37     | 33   | 65    | 48   |

nitrogen. Naturally, the actual loss of protein through the breast milk comes into consideration here. Urea is slightly low in both periods, though post-partum it evidences a possible beginning recovery to the usual normal levels. This is no more than yet another report of the low urea which has come to be

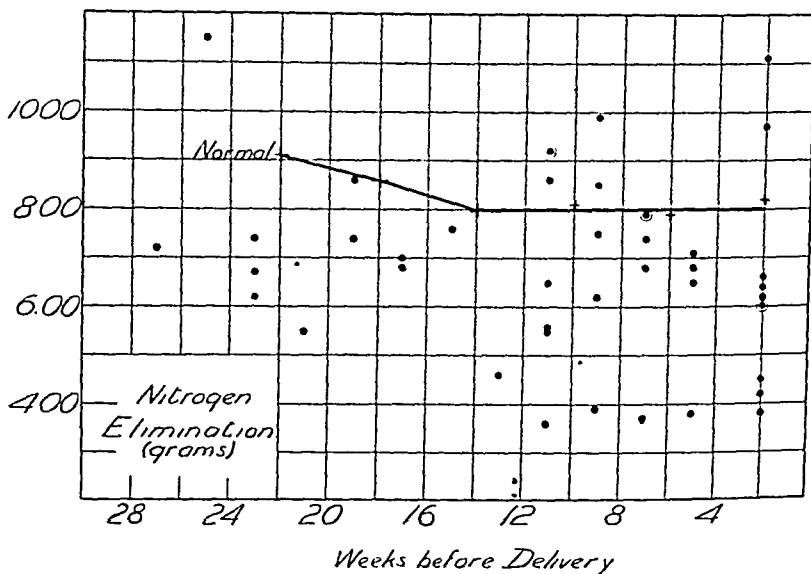


Chart 3.

recognized as a normal feature of pregnancy. A high uric acid during pregnancy shows a drop to a more normal level when the strain of gestation is removed; ammonia is slightly but definitely high; the average for residual nitrogen is well within the normal range, but about one-third of the series show values which are absolutely high.

In the toxic group, several differences at once present indicating the general disturbance of metabolism associated with the toxic status. The total nitrogen is significantly lower. Even though this could result from an impaired appetite and lowered food intake it would still be an evidence of toxicity. To render this depression more apparent, the individual measurements are plotted in Chart 3. The curve marked "normal" derives from the average data of the control series and serves as a delimiting boundary. But few of the observations fall above its level, while the great majority are significantly below.

Of the other figures, urea is still lower, uric acid still higher, while the residual fraction is frankly above the liberal allowance for the normal, and two-thirds of the group participate in this departure. With the emptying of the uterus, the nitrogen rises to a level normal in relation to the control group, urea rises relatively and absolutely, and the residual nitrogen falls both in amount and in the frequency of report of high levels. As these women were examined during the fortnight following delivery, the time factor bulks large and the trend toward normality becomes even more significant of a changed status. Ammonia is lower in the toxic than in the normal series. In the postpartum period it more closely approaches the conventional normal than does that in the control series. Loss of fixed alkali through the milk should affect both groups equally and the explanation of the anomaly is not directly forthcoming. The originally high uric acid shows a slight downward trend but remains at a height which is frankly above the normal. Probably this reflects the hepatic factor present in so many of the cases; hepatic dysfunction is known to modify purin metabolism, and these cases present a functional derangement which but slowly returns to the normal. The liver complement in the picture could also account for the high residual nitrogen figures.

To summarize this section briefly, pregnancy as such, produces changes in the urine nitrogen suggestive of some degree of toxemia, while actual toxicity during this condition accentuates these changes. Further, with the removal of the common causal factor of gestation by delivery, even in the toxic group there is evidence of a prompt retrograde movement toward normal relationships.

The various dissolved nitrogen-containing substances in the blood usually designated as the "nonprotein nitrogen" are, with but one significant exception, ammonia, the forerunners of the urine nitrogen in its many subdivisions.

In reviewing what may be designated as the "nitrogen partition" of the blood, only the ammonia is lacking from the group. Convention and propriety further dictate a different method of recording the blood constituents to express more significantly the meaning of departures from the normal. The urine is a summation of the various secreting levels of the twenty-four hours during which, by thermodynamic work, the blood is concentrated to form the urine. This collects in the bladder, a container inside the body, and is emptied from it at intervals into other containers only relatively more remote from the secreting agent. The blood, on the other hand, is the channel of ingress and egress of foodstuffs and wastes, and the levels of its several constituents are maintained at a relative constancy through the agency of the vast number of individual factors contributing to the continuance of homeostasis. Under the force of superimposed effects, new equilibria are established at other levels but the tendency always is to return to the earlier conditions when the extraneous agencies cease to operate. If the pressure be not too great, the homeostatic influences tend to compensate for such effects and restore or maintain normal levels even during the continued operation of the added effect. Temporary changes in blood sugar levels during sugar absorption

and the maintenance of blood  $P_{II}$  in compensated ketosis are typical illustrations. The absolute levels in the blood thus assume a significance that in the urine is largely absorbed by the single factor of the total nitrogen. The average blood levels ante- and postpartum in the two series are collected in Table II.

TABLE II  
BLOOD NITROGEN

| DATUM                     | NORMAL |      | TOXIC |      |
|---------------------------|--------|------|-------|------|
|                           | A.P.   | P.P. | A.P.  | P.P. |
| Nonprotein nitrogen (mg.) | 25     | 32   | 28    | 32   |
| Urea nitrogen (mg.)       | 12     | 16   | 14    | 15+  |
| Uric acid (mg.)           | 3.3    | 3.7  | 3.6   | 3.6  |
| Creatinine (mg.)          | 1.5    | 1.5  | 1.5   | 1.5  |
| Residual nitrogen (mg.)   | 11.3   | 14.2 | 12.2  | 15.2 |

It has long been a well-recognized fact that the nonprotein nitrogen and its composing fractions are, in the main, definitely depressed during pregnancy. We have never been able to record the sharp additional decline in blood urea to levels approaching 30 per cent of the whole, reported by several observers; we have uniformly found the values in pregnancy to coincide with those in normal males and nonpregnant females, or from 45 per cent to 50 per cent. In the present instance both series show an entirely normal relationship. On the other hand, uric acid values show a modest upward tendency, although in normal pregnancy individual values may fall within the conventional normal limits. In relation to the nonprotein nitrogen, however, the uric acid values are definitely high. It is apparent that antepartum all of the values, barring creatinine and uric acid, are definitely low, and that delivery engenders a prompt return to normal levels. In the toxic series, the values antepartum are all somewhat higher, a presumptive evidence of low-grade retention that is in harmony with the recognized renal influence in this condition. After delivery the blood reverts to normal levels that are very close to those of the normal controls. Recognizing that the homeostatic agencies operate most effectively in the blood stream and that existing chemical methods are incompetent to record very small variations, the blood levels fail to give evidence of that slower return to normal levels that is clearly shown by the urine data from the toxic group.

To summarize briefly, pregnancy causes a lowering of the nitrogenous constituents of the blood, uric acid excepted, which is less marked in cases of toxemia as the renal element here introduces an opposing influence. Both normal and toxic pregnancies tend to revert to the usual blood levels shortly after delivery.

#### CONCLUSION

1. The blood and urine data reflecting the protein metabolism have been determined in series of normal and toxic pregnant women.
2. Characteristic urine findings are recorded with pregnant patients; the hepatic and renal features of toxemia are superimposed influences adding their special effects to those of the basic condition.

3. Termination of pregnancy initiates a return to normal levels, the latter less rapid in the toxic group.

4. Blood nitrogen levels are low in normal pregnancy. Toxemia tends to develop the renal influence as an opposing agency.

5. Restoration to normal blood levels follows rapidly on the termination of the pregnancy in both study groups.

The authors express their indebtedness to all who have participated in the study.

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## VERRUCOUS AORTITIS WITH SPECIAL REGARD TO ANEURYSM FORMATION IN CHILDREN\*

BENJAMIN H. NEIMAN, M.D., CHICAGO, ILL.

FOR a long time, nonsuppurative inflammatory lesions in the aorta were attributed chiefly to syphilis, a conception first expressed by Doehle in 1885 and later in 1899 by Heller.<sup>1</sup> The "Doehle-Hellersche Aortitis" held sway over medical thought until more recent investigations revealed that acute infectious diseases as typhoid fever, typhus, influenza, and scarlet fever, may also cause inflammatory changes in the aorta (Siegmond<sup>2</sup>). Of particular importance, however, is the fact that rheumatic fever seems quite frequently to leave its mark on the large arteries (Klotz,<sup>15</sup> Pappenheimer and von Glahn<sup>16</sup>).

Although inflammatory changes in the aorta were suspected in the course of rheumatic fever, no sustained effort to define the histologic features was made until the work of Klotz<sup>15</sup> in 1912. Clinically, the diagnosis has been made many times, but it is unverified by autopsy.

To this category of unverified diagnoses belong the reports of Bouillaud<sup>3</sup> (1840), Roger<sup>4</sup> (1863), LeLong<sup>5</sup> (1869), Lauenstein<sup>6</sup> (1876), Leger<sup>7</sup> (1877), Marfan<sup>8</sup> (1901), Méry et Guillemont<sup>9</sup> (1902), La Rue<sup>10</sup> (1903), Renon<sup>11</sup> (1905), Weil et Menard<sup>12</sup> (1912). Bernert<sup>13</sup> (1910) deserves the credit for calling at-

\*From the Department of Pathology, Cook County Hospital, Dr. R. H. Jaffé, Director.  
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tention to the fact that many of the previously reported cases, cited as rheumatic in origin, were probably examples of mycotic aneurysms. Rabé<sup>14</sup> (1912) was the first to present histologic findings.

In 1912 the first serious effort was made by Oskar Klotz<sup>15</sup> to study the histologic lesions in the aorta occurring as a result of rheumatic infection. In 15 cases of rheumatism which came to necropsy, he had 3 patients, aged nine, sixteen, and twenty years, dying during the first attack of rheumatic fever; 4 cases of patients, aged twenty-eight, thirty, thirty-six and forty-four, with history of recurrent attacks; and 8 cases of patients with chronic rheumatism, all of whom had suffered from recurrent or progressive heart disease associated with rheumatic fever. He concludes that there appears to be a fairly definite form of arterial disease which is associated with rheumatic fever in its different stages. The arteries react to the irritant in a true inflammation, and this reaction is observed in the adventitia and the outer portion of the media. In the acute stages, the inflammatory exudate is of the nonsuppurative variety in which the lymphoid cell is most prominent. The inflammation occurs particularly in the neighborhood of the smaller arteries, while the medium-sized vessels are little affected. On the other hand, the larger arteries, which are supplied in their outer coats by nutrient vessels, are damaged by the inflammatory process, which travels along the vasa vasorum. He observed, that during the acute process, the changes in the aorta consist of a destruction of tissue noted in the loss of muscle and elastic elements. Similar degenerations are common in the heart. At times the degenerative processes are extensive, leading to the loss of considerable tissue in, and probably weakening of, the media. The intima of the arteries, he states, does not appear to be primarily affected. In the early stages, slight superficial fatty changes are sometimes noted, while later in the chronic stages the intima proliferates, giving rise to a nodular endarteritis.

In 76 cases of acute rheumatic fever, Pappenheimer and von Glahn<sup>16</sup> found the distinctive lesion of the aorta to be a scar, roughly the shape of an inverted wedge, often acellular and located in the region of the nutrient vessels of the media. This type of arterial lesion was not found in a comparative study of 77 nonrheumatic cases, with the exception of one, in which death resulted from pneumococcal meningitis. In the aortic adventitia, they found specific changes consisting of the presence, in some cases, of typical Aschoff cells, showing, however, little tendency for grouping. These cells, they regard as diagnostic. They are rounded or oval. The cytoplasm is basophilic and stains intensely with pyronin. The nuclei each have a distinct nucleolus, and the cells seem to be arranged about degenerating collagen fibers. In their second communication on this subject, the authors described earlier reactions in the media to support the view that the perivascular medial scars are the sequelae of an earlier focal perivascular inflammatory lesion.

Following the first paper by Pappenheimer and von Glahn, many communications began to appear, both in American and European literature, giving histologic evidence of destructive effects of rheumatic fever on the aorta (Weisl,<sup>29</sup> Besançon and Weil<sup>30</sup>), even so severe as to lead to aneurysm formation (Gray and Aitken<sup>31</sup>).

Changes in the adventitia of the aorta were further emphasized as perivascular round cell accumulations and nodular fibrosis (Chiari,<sup>18</sup> Barnard<sup>20</sup>). Clawson<sup>21</sup> injected *Streptococci viridans* intravenously into monkeys and produced changes in the media and adventitia similar to those seen in rheumatic fever. He also gave subcutaneous administrations of the cocci to rabbits but produced only subcutaneous nodules similar to those described by Swift<sup>22</sup> and Frank.<sup>23</sup>

Sacks,<sup>24</sup> in his review of the pathology of rheumatic fever, states that since the vasa of the pulmonary artery are derived from the coronary arteries, lesions in this artery are to be expected. Kugel and Epstein,<sup>24</sup> on the basis of this supposition, examined a series of 24 cases of rheumatic fever and found 5 in which there were histologic evidences of active inflammatory changes in the pulmonary artery. The changes in the pulmonary artery were of two types; namely, a diffuse cellular infiltration in the intima and subintimal layers of the media and focal perivascular accumulation of cells comparable to the Aschoff nodule in the myocardium. In 2 cases, there were macroscopic changes in the intima consisting of small, raised, dull gray yellow patches of irregular outline. In 2, there was a disruption of the elastic fibers of the media, almost as conspicuous as in syphilis. In 14 cases, the pulmonary valve was involved showing an interstitial valvulitis. In 3 cases, Aschoff bodies and in 6 verrucae were found. In 17 cases, in which there were changes in the musculoarterial junction, Aschoff bodies were found in a few. The aorta was involved in 22 cases. In 4 cases, gross lesions in the intima were present. As controls, they used 31 cases in which there was a nonrheumatic pericarditis, and in 75 cases no pericarditis or rheumatic cardiac disease was present. They found nothing in any of the controls which was in any way confusing.

Destructive lesions in the media with extensive scarring were also reported by Paul,<sup>27</sup> in the course of rheumatic fever, and Wätjan<sup>26</sup> observed Aschoff bodies in the epicardial fat over the anterior surface of the pulmonary artery. Gotthardt<sup>25</sup> reported a case of verrucous endocarditis of all the valves and fibrinous pericarditis which were accompanied by an endarteritis of the pulmonary artery. Chiari<sup>19</sup> found a fibrosis of the adventitia of this artery.

#### PRESENTATION OF CASES

In a series of 4,100 consecutive autopsies, 3 cases have been found in which there was a verrucous lesion on the intima of the aorta.

CASE 1.—An Italian female child, eleven years old, entered the Cook County Hospital on March 8, 1932. She was apparently well until two weeks prior to entrance when she developed pain over the heart, cough, and fever. On the day of entrance into the hospital, she had a moderate nose bleed.

The birth and development history was negative.

The child had had measles and chickenpox.

On physical examination, the patient was found to be well nourished but somewhat undersized. The temperature was 102.4° F.; pulse, 124; respiration, 28; and blood pressure, 140/60. The skin was dark and there was long, dark hair over the upper lip and along the side of the cheek, on the back and a little over the lower extremities. The pubic hair was luxurious and of female distribution. There were diffuse coarse râles heard over both lung fields.

The heart was enlarged transversely and there was a soft, systolic thrill and a systolic and diastolic murmur over the apex. Over the aortic area, indistinct murmurs were heard.

The pulse at the wrist was forceful, of high pulse pressure, quickly striking the finger and receding. The carotid pulsations were easily visible. The femoral, popliteal, dorsalis pedis, and posterior tibial arteries were not palpable, nor could they be detected by the blood pressure apparatus.

The liver was palpable, but the spleen was not.

The white blood count was 20,100 with 84 per cent polymorphonuclear leucocytes. There were many macrocytes present. Polychromatophilia and anisocytosis were evident. The urine was negative as was also the blood Wassermann.

The clinical diagnosis was endocarditis of the aortic and mitral valves, diffuse bronchopneumonia, and coarctation of the aorta.

While in the ward, the patient was rather comfortable but ran a low grade remittent fever of 99° to 100° F. The findings in her lungs spread gradually. About one week after entering the ward, the child was seen sitting up in bed and resting quietly but coughing frequently. Forty-five minutes later, she was found on the floor dead.

*Autopsy.*—(Performed by Dr. R. H. Jaffé.) The essential findings were in the heart, aorta, and incidental interesting findings in the genitalia.

The heart weighed 250 gm. The wall of the left ventricle measured 12 mm., and that of the right ventricle 5 mm. in thickness. The epicardium was thin, transparent, smooth, and shiny. The apex was formed by the left ventricle. The myocardium was purple, gray and friable. The transverse and vertical diameters of the left ventricle were 8.5 cm. The trabeculae and papillary muscles were slightly flattened. On the auricular surface of both cusps of the mitral valve, were present pinhead-sized, granular, firm, and slightly transparent nodules. The free edges of the aortic leaflets were thickened and rounded, and along the limbus there were firm, sandlike, granular deposits present.

The aorta measured 52 mm. in circumference above the valve. About 1 cm. above the aortic valve, there was an oblique, transverse tear which was 35 mm. wide. The edges of the tear were indented, and 2 mm. longitudinal bridge of intact wall separated the tear into a larger right and smaller left portion. The floor of the tear exposed a thin, pink gray membrane with fine, longitudinal lines and small, granular elevations. The adventitia was injected. To the right, the tear terminated in an irregular, slightly depressed, light brown area. In the ascending aorta, there were several up to 25 × 4 mm. streaklike, light yellow areas. Just beyond the origin of the left subclavian artery, the lumen of the aorta becomes suddenly narrowed to a diameter of about 1 mm. Beyond the narrowed portion, the circumference of the aorta widened to about 58 mm. Small, light yellow plaques were found in the upper wall of the arch. At the diaphragm, the circumference of the aorta was 36 mm., and below the origin of the renal arteries it was 22 mm.

Above the bifurcation of the aorta, there was a worm-shaped, loose, cylindrical, light pink gray blood clot 55 mm. long and 10 mm. in transverse diameter. The lower end of the blood clot projected into a flagellum-like extension 35 mm. long. The upper end terminated into a pointed projection 3 mm. long. The circumference of the aorta above the bifurcation was 18 mm. The internal mammary artery had a circumference of 6 mm.

The uterus measured 45 by 25 by 8 mm.

*External genitalia:* The clitoris was about twice the normal size. The vestibulum vaginae and vagina were well developed. The vulva appeared rather narrow because of an enlargement of the labia minora.

*Ovaries:* The right ovary was replaced by an ovoid body, 18 by 10 by 6 mm. in diameter, which showed a distinct, light pink yellow capsule, and on sectioning, was found to be composed of a finely granular, light yellow tissue. The left ovary was missing, and in its place was found a ridge 25 mm. wide and 3 mm. high.



## Microscopic examination:

**Aorta:** The tear in the ascending portion of the aorta involved the intima and the entire media and exposed the adventitia which was densely infiltrated by polymorphonuclear leucocytes. Approaching the tear, the intima became slightly thickened, and in the region of the tear, was everted, covering the edges of the torn structures. The thickening of the intima resulted from a cellular accumulation between endothelium and internal elastic membrane. Delicate elastic fibrils were separated by swollen fibrocytes with large, pale nuclei, small round cells, and histocytic elements with an ample cytoplasm and round or oval nuclei. There were bizarre-shaped, drawn out nuclei free between the intact cells. The internal elastic membrane was split into several layers and in the everted portion showed areas frayed to the extent that it was completely interrupted. Near the torn portion, the muscle and elastic fibers of the media passed rather abruptly into a zone of complete necrosis without structural differentiation and scanty chromatin granules. The necrotic area stained from brass yellow to deep yellow brown after van Gieson and pink red with eosin. In the outer portion of the media, single mononuclear elements were wedged in between trabeculae of necrotic tissue,



Fig. 1.—(Case 1.) Aorta. Section taken through the area of rupture. Elastica stain ( $\times 100$ ).

and the necrosis extended for a short distance beyond the tear along the external elastic membrane. In the floor of the defect, there were only a few small segments of the external elastic membrane visible.

Between the polymorphonuclear leucocytes which infiltrated the adventitia, were found single, large, degenerating fibrocytes, the nuclei of which could assume very irregular forms. Elastic fibrils were practically absent.

Away from the tear, the intima appeared well preserved. In the media, there were circumscribed areas of increase of the metachromatic substance which spread apart the elastic elements. Near the outer elastic membrane, there were small areas in which the coarse elastic fibers seemed to have disappeared while the fine fibrils still could be recognized. The adventitia was of distinctly increased cellularity. The fibrocytes were swollen, often separated and mobilized. There were many mononuclear cells with a distinct rim of cytoplasm, and lymphocytes and a few oxyphilic leucocytes. In addition to these cell forms, elongated cells were found with pyknotic, crenated and elongated nuclei, which could be seen also between the muscle fibers of the adjacent portion of the media.

**Myocardium:** There were in the myocardium, numerous nodular accumulations of large cells which were located near the larger blood vessels. The cells which formed the nodules

had a pale stained cytoplasm and oval nuclei with a central indented spindle of chromatin. These cells centered about areas of fibrinoid necrosis. Between them, a few lymphocytes and leucocytes were found. Underneath the endocardium, areas of necrosis were found, which were surrounded by cells of histocyte type distinctly different from the cells of myocyte type. In addition to the cellular nodules, there were flame-shaped scars radiating between the muscle fibers, which were increased in thickness and the striations of which were obscured.

The anatomic diagnosis was coarctation of the aorta with thrombosis of the aorta distal to the coarctation; spontaneous rupture of the aorta above the aortic valve and rheumatic aortitis with necrosis of the media; verrucous endocarditis of the mitral and aortic valve and slight fibroplastic deformity of the aortic valve; slight excentric hypertrophy of the heart; moderate dilatation of the internal mammary and intercostal arteries; chronic passive congestion of the lungs, liver, spleen and kidneys; bronchopneumonia of the right lower pulmonary lobe; bilateral hydrothorax; follicular hyperplasia of the spleen; pseudoherniaphroditismus internus masculinus.

CASE 2.—A colored female, twenty-seven years old, entered the Cook County Hospital on April 23, 1932, stating that she had been well until one month ago. At this time, she began having periodic attacks in which her heart appeared to "jump." These attacks were followed by precordial boring pain that radiated to her left shoulder down to her left arm and seemed to stay there. The attacks were easily precipitated by any exertion or excitement. At the onset the attacks lasted but a few minutes, but recently had increased in length up to twenty minutes or more. Often during the attack, the patient fainted, but usually she had to stop immediately in her work. There was no great fear of death at any time. Occasionally nausea, vomiting, and urinary urgency followed an attack. The patient was married twice and received three injections of mercury in her buttocks in 1922.

The physical examination revealed a well-nourished colored female, lying quietly in bed and not acutely ill. The temperature was 98.8° F.; pulse, 80; respiration, 20; and the blood pressure, 106/64. The pupils reacted to light and accommodation. The heart was not enlarged; there were no murmurs or thrills. The rhythm was regular. The chest and abdomen were essentially negative. The red blood count was 3,260,000; white blood count, 7,800; and the hemoglobin 55 per cent.

The patient had several attacks during her short stay in the hospital, some slightly alleviated by amyl nitrate, others by quinidine. On the morning of the day of her death, the patient had a typical attack, which was as follows: She was sitting up quietly in bed when she suddenly toppled from her back rest, became livid, cyanotic, stopped breathing, and then broke out into a cold sweat. The pulse was imperceptible, even by auscultation. Artificial respiration was administered for five minutes and adrenalin and caffeine injected. The patient rallied but four hours later died during a similar attack. Blood for a Kahn test could not be taken during life because the prick of the needle initiated an attack. The postmortem blood Kahn reaction was 2+ positive.

*Autopsy.*—(Performed by Dr. R. H. Jaffé.) The essential pathologic findings were in the heart and in the aorta.

The circumference of the aorta was 56 mm. at the valve, 30 mm. at the diaphragm, and 26 mm. at the bifurcation. In the ascending part of the aorta, above the valve, there were light yellow, slightly raised patches. Above the commissures, were gray white, 10 mm.—areas covered by fine, sandlike, granular, light gray precipitates. These precipitates extended above the right leaflet for a distance of 20 mm. In the region of the arch, there were a few whitish areas and also a slightly raised, 10 mm., pale yellow gray plaque covered by fine granular precipitate. Throughout the descendens portion of the aorta, there were many small, light yellow plaques.

The heart weighed 220 gm. The left ventricle measured 15 mm. and the right ventricle 5 mm. in thickness. The epicardium was smooth and studded with pinpoint-sized, light pink gray patches. The myocardium was light purple pink and friable. The ventricles were dilated and the trabeculae were flattened.

### Microscopic:

**Heart:** In the inner third of the myocardium, there were irregular areas of severe alteration of the muscle fibers. The fibers had lost all structural differentiation and appeared as homogeneous bands. The nuclei were broken up into chromatin granules or were entirely missing. Between these muscle fibers could be seen a moderate number of polymorphonuclear leucocytes, and around the adjacent larger vessels were larger accumulations of these cells. Throughout the wall, many muscle fibers contained fine lipid granules. In the epicardium, there were a few small, round cell accumulations.

**Aorta:** Supravalvular verrucous area. The intima of the aorta was covered by a layer of fibrinoid material with elongated, drawn out and segmented, deeply stained nuclei. Underneath this layer, the intima was thickened and cellular. Here many fibrocytes, young, smooth muscle fibers, small round cells, and neutrophilic and oxyphilic leucocytes were found. In the center of the area, the changes terminated in a large focus of necrosis which involved not

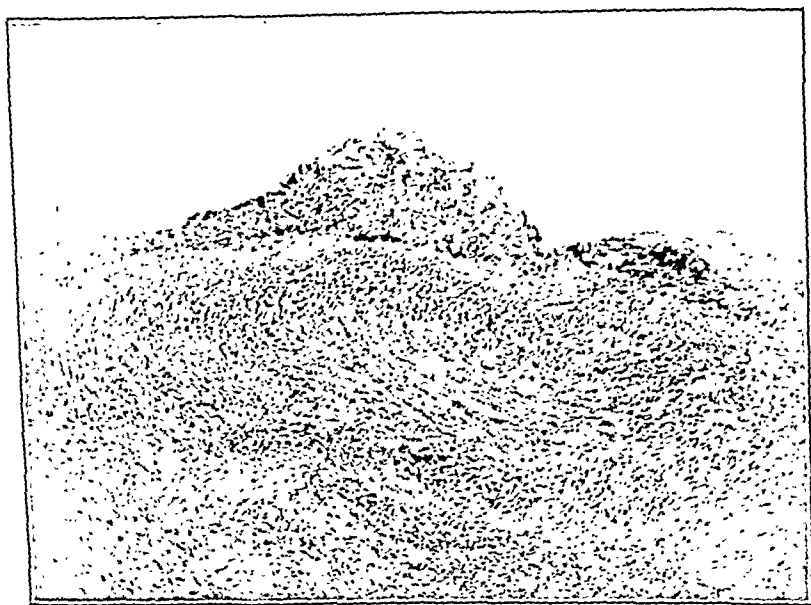


Fig. 2.—(Case 2.) Aorta. Fully developed verruca. Hematoxylin-eosin stain (X150).

only the thickened intima, but extended also deep into the media. On the inner surface, the necrotic tissue fused with the fibrinoid layer, the point of fusion being marked by streams of degenerated pus cells with karyorrhectic nuclei. Deeper down, the necrotic material showed early calcification. In the periphery of the necrosis, were found focal accumulations of pus cells; these continued beyond the necrosis between the muscle fibers of the media which here, too, were severely degenerated and partially necrotic, while the elastic fibers were swollen up and disintegrated.

Underneath the necrosis, at the border between the media and adventitia, there was a lipid granuloma composed of pseudoxanthomatous cells, plasma cells, and small round cells, and of a few oxyphilic leucocytes. From this granuloma, tufts of cells extended into the media. In addition to the large area of necrosis, there were several smaller ones which were located in the deeper portions of the intima and in the media. They showed fine calcium deposits and were not separated from the surrounding tissue. Underneath these areas, there were perivascular accumulations of round cells, plasma cells, and eosinophilic leucocytes. The vasa vasorum had greatly thickened walls, and there were many dense accumulations of lymphocytes and plasma cells in the adventitia.

**Plaque in the Arch of the Aorta:** The inner surface of the plaque was covered by a thin layer of fibrinoid material which took the Weigert stain. Underneath this layer the intima was thickened and cellular. The fibrocytes were swollen and increased in number, and there were loosely scattered neutrophilic and oxyphilic leucocytes, small and large lymphoid round cells, and focal accumulations of plasma cells. In the center of the plaque, a large area of necrosis was present, which extended into the media without a sharp line of demarcation and contained fine calcium granules in the basal parts. From the fibrinoid surface layer, the necrotic tissue was separated by a strip of living tissue upon which followed a collection of disintegrating polymorphonuclear leucocytes with broken up and pyknotic nuclei. Bacteria could not be demonstrated in this area or in the necrotic tissue itself. In the region of the necrosis, the internal elastic membrane was completely destroyed, the destruction being preceded by a splitting into the finest fibrils. Throughout the media, there were many newly formed capillaries extending close to the intima and surrounded by lymphocytes and plasma cells. The piercing of the media by these vessels and infiltration was associated with a disappearance of the elastic fibers. The adventitia was thickened and fibrosed, and the arteries of the adventitia showed a very marked proliferation of the intima with much narrowing of the lumen. There were many focal accumulations of plasma cells and lymphocytes.

**Midportion of Aorta Ascendens:** There was a thickening of the intima with intra- and extracellular lipid deposits in the deeper portions. The internal elastic membrane was split and accumulations of fat-filled cells were present between the separated elastic structures. The capillaries, beyond the outer third of the media, were proliferated and surrounded by a slight round cell infiltration. There was a very pronounced fibrosis of the adventitia with a thickening of the walls of the vasa vasorum, and large accumulations of lymphocytes and plasma cells were present in the adventitia.

Levaditi's stain revealed many argentafin granules but no spirochetes in the necrotic tissue.

The anatomic diagnosis was verrucous aortitis; syphilitic aortitis; acute myocarditis; passive congestion of the spleen, liver, and kidneys; adhesions about the liver, spleen, and both ovaries.

**CASE 3.**—A four-year-old white female child was brought into the Cook County Hospital on July 21, 1932. The mother stated that the child was well until two weeks before entrance when it developed colicky pain in the abdomen. It was also noticed that during the past two weeks the child had had a fever in the latter part of the afternoon. Development history was normal. The mother had had no miscarriages.

The physical examination revealed a well-developed child. The temperature was 103.4° F.; pulse, 140; respiration, 26. The heart was enlarged to the left. The apex beat was displaced slightly to the right. Upon examination of the chest, the breath sounds were found to be diminished in the left midaxillary line with impairment of resonance over the left apex anteriorly. The white blood count was 29,400 with 78 per cent polymorphonuclear leucocytes. The x-ray revealed a very marked cardiac enlargement.

The clinical diagnosis was pericarditis with effusion. Five days after entrance into the hospital, a pericardial paracentesis yielded 35 c.c. of a straw-colored fluid. About ten minutes later, the child became cyanotic and expired.

**Autopsy.**—(Dr. R. H. Jaffé.) The pericardial sac extended from the right mammary line to the left anterior axillary line. The sac contained 250 c.c. of coagulated blood. The heart measured 70 mm. in vertical and 50 mm. in transverse diameters. The surface was covered by loosely adherent, granular light pink gray membranes. The wall of the left ventricle was 11 mm. and that of the right ventricle, 5 mm. in thickness. The myocardium was light purple pink and friable. The heart valves were grossly unchanged.

**Aorta:** At the valve, the aorta measured 42 mm.; at the diaphragm, 28 mm.; and at the bifurcation, 18 mm. in circumference. The intima above the valve was smooth. Beyond the arch of the aorta, 8 mm. below the opening of the left subclavian artery, located in the anterior wall, there was an oval opening 7 by 5 mm. in diameter. The edges of the opening were smooth and slightly everted. The intima was smooth except for fine, pinpoint-sized granular and slightly transparent deposits near the lower edge. The opening led into a sac

which was 7 cm. in vertical, 5 cm. in transverse, and 5 cm. in anteroposterior diameters. The sac, which was adherent to the median aspect of the left upper pulmonary lobe, bulged into the pericardial sac stretching its reflexed portion. The sac compressed slightly the left branch of the pulmonary artery. At the greatest convexity of the intrapericardial portion of the sac, there was an irregular hole 25 by 20 mm. in diameter, with indented edges. In the sac, which was lined by a whitish finely granular membrane, there was a small amount of loosely coagulated blood.

#### Microscopic examination:

**Aorta:** When the opening in the descending aorta was approached, the first change visible was the appearance of a layer of fibrinoid material which rested upon the internal elastic membrane. This fibrinoid material contained irregular, deeply stained, and shrunken nuclei. Near the opening, the layer was raised to nodular elevations which stained brass yellow after the van Gieson method. Here and there close to the internal elastic membrane, a group of better preserved oval nuclei was found forming small palisades with the nuclei



Fig. 3.—(Case 3.) Aorta at point of rupture. Hematoxylin-eosin stain ( $\times 150$ ).

perpendicular to the membrane. At the opening, the layer of fibrinoid necrosis bent outward to pass into the internal lining of the sac. The point of bending was marked by a greater cellularity, and besides cells with shrunken and indented nuclei, there were some lymphocytes and an occasional multinucleated giant cell. Underneath the nodular elevations, the media revealed very severe changes. The muscle fibers were necrotic, hyalinized, and the chromatin granules of the broken down nuclei formed fine, dustlike precipitates. At the border of the media and adventitia, the zone of necrosis extended for some distance beyond the changes of the intima in the form of a tongue-like projection. Stains for elastin demonstrated that the internal elastic membrane was destroyed underneath the nodular formations of fibrinoid material. In the necrotic area of the media, the coarser elastic fibers were still visible, but they were broken up into small pieces and curled, and the finer fibrils had disappeared. The adventitia was well preserved and contained only an occasional small accumulation of round cells.

The wall of the aneurysm was in continuation with the wall of the aorta, the layers of which could be recognized though they were severely changed. Innermost, there was a broad band of fibrinoid necrosis with shrunken, pyknotic, and broken up nuclei, which band cor-

responded to the intima and media. Immediately adjacent to the lumen, remnants of intact tissue, which caused the internal lining of the sac to be smooth could be detected. These remnants consisted of a few connective tissue trabecles with preserved fibrocyte nuclei. The connective tissue soon passed into the fibrinoid necrotic material which was particularly rich in nuclear debris in the places corresponding to the media. In these places, remnants of elastic tissue in the form of tortuous segments were found still suggesting the arrangement of the elastic tissue of the media. Near the adventitia, mononuclear cells, neutrophilic, and a few oxyphilic leucocytes were seen between the shrunken and disintegrated nuclei of the preexistent structures.

The adventitia was the only layer which had remained intact. It was very thick and was formed by dense connective tissue interspersed by a moderate number of elastic fibrils. There were many dilated capillary blood vessels present, which were often surrounded by

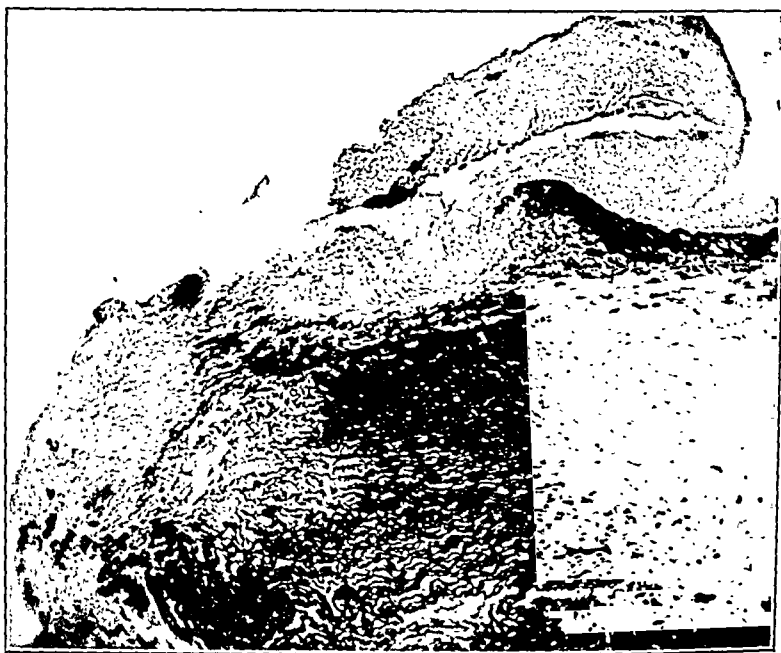


Fig. 4.—(Case 3.) Aorta at point of rupture. Elastica stain ( $\times 150$ ).

accumulations of lymphocytes, plasma cells, histocytes, and a few leucocytes. Gram-Weigert stain demonstrated single gram-positive diplococci and streptococci in the nodular elevations on the inside of the aorta. These cocci became abundant in the region of leucocytic-histocytic infiltrations of the aneurysmal sac where they were found also inside of mononuclear and polymorphonuclear elements.

Sections taken from other portions of the aorta did not show any abnormal changes:

**Myocardium:** About the blood vessels of the myocardium were small scars which radiate between the muscle fibers and contain an occasional small accumulation of myocytes.

The anatomic diagnosis was verrucous streptococcic aortitis of the descending portion with formation of an aneurysm; perforation of the aneurysm into the pericardial sac and tamponade of the pericardial sac by the hemorrhage; compression atelectasis of the left lung and recent hemorrhagic infarct in the apex of the left lung; loose, fibrous adhesions about the left lung; compensatory emphysema of the right lung; cloudy swelling and anemia of the liver; hyperplasia of the lymph follicles of the spleen; slight congestion of the kidneys; ascites and left hydrothorax.

## DISCUSSION

The changes in the adventitia and media of the aorta, in the course of rheumatic infection, have been much emphasized to the neglect of the important changes that may involve the intima. Few references to the occurrence of verrucous lesions in the intima of the aorta and large arteries are present in the literature. Heydloff<sup>12</sup> described verrucae in the innominate and carotid arteries. In one case, that of Barbacci,<sup>13</sup> these lesions begin 1 cm. above the sinus of Valsalva and histologically were not unlike the organizing verrucae of rheumatic endocarditis. Bacteria could not be demonstrated in them. The heart was normal.

It is quite possible that some of the cases were rheumatic in origin though no reference is made to this possibility by any of the authors. Neither are the histologic descriptions sufficiently detailed to justify any definite conclusions. It must be remembered, however, that these papers antedated the recognition of the specificity of the lesions in rheumatic cardiac disease. While many of the older writers, particularly the French authors, have ascribed to the "rheumatic virus" an important place in the causation of arterial disease, the connection was based upon clinical inference rather than histologic specificity.

Pappenheimer and von Glahn<sup>16</sup> recognized the possibility of invasion of the aorta by way of the lumen, as did also Perla and Deutch<sup>21</sup> and Kugel and Epstein.<sup>24</sup> Its importance, however, as the primary port of entrance has not been sufficiently stressed. Where mention of this possibility is made, it is considered unlikely for want of sufficient evidence.

In a consideration of the various types of inflammatory lesions described in the aorta in rheumatic fever, three groups may be distinguished. First, invasion by means of the vasa vasorum with involvement of the adventitia alone; more extensive inflammatory reaction may lead to involvement of the media or even to the intima and thus severe damage to all three layers. Should the acute reaction, described as the forerunner of the flame-shaped scars (Pappenheimer and von Glahn<sup>16</sup>), be a little more extensive or should several of these lesions be localized in one area, the resulting healing process may terminate in a gross scar; or should the reaction take place within a wall of the vasa vasorum with a subsequent thrombosis of that vessel, gross scarring may result. The rarity of these gross lesions in the aorta may be attributed either to the relatively slight involvement of the aorta in rheumatic fever, or to the belief that because the scars in the media resemble a process produced by syphilis, it is a syphilitic process. Aside from the history, the clinical picture, the serologic test, and the observations in other parts of the body, there are definite histologic differences between the scars of the media in syphilis and the scars found in cases in which they are believed to be due to rheumatic fever (Gray and Aitken<sup>21</sup>). Should the resultant scars or the degenerative and inflammatory process preceding these scars weaken the media to such an extent as to be unable to support the burden of the passing blood stream, a break results with a subsequent stretching of the remaining portions of the wall of the aorta, and an aneurysm forms.

Second, we may have invasion of the aorta by direct extension from an adjacent lesion (Pappenheimer and von Glahn<sup>17</sup>).

Third, we may have invasion from the lumen with the primary port of entrance in the intima with the formation of verrucae.

In the three cases presented here, we found one type of lesion common to all, namely verrucae. In Case 3 streptococci were demonstrated. In one case an aneurysm was present and in another, a rupture of the aorta. In attempting to link up the findings in these cases, we are struck with the common feature of all. The histologic picture of the changes in the intima are very similar to the changes observed in the formation of the nodules throughout the body in the course of rheumatic fever. Klinge<sup>35</sup> looks upon the widespread connective tissue damages as of close morphologic relationship. According to him, the first and essentially evident change consists, not in the cellular nodules, which are already developed, but as a degenerative change of the ground substance of the connective tissue. In a circumscribed area at the point of attack of the infecting agent, a fibrinoid swelling of the intima takes place; this is the first reaction. This stage, which Klinge<sup>35</sup> calls the early rheumatic infiltration (*rheumatisches Frühinfiltrat*), is found in the first fourteen days of the affection. It is characterized by an edema of the connective tissue and in the single bundles of fibrils; wax-like refractive masses are seen with the fibrils failing to stain with ordinary methods. When the damage is very severe, necrosis results.

In the light of these findings, we can interpret the formation of the verrucae as follows: first, there is a fibrinoid swelling, in a circumscribed area of the intima, at the point of attack of the infecting agent. The fibrinoid swelling progresses to such an extent that it rises above the surface of the unaffected intima. Later, it is followed by a proliferation of the cells of the intima so that a definite firm nodule results. The proliferating cells are chiefly of fibrocyte type with a varying amount of small round cells. The affection may either subside leaving in its wake a plaque, or it may progress, when regressive changes in the form of necrosis become more prominent. The inflammatory and necrotic changes may advance into the deeper layers breaking through the internal elastic membrane and involving also the media. The destruction in the media may be so extensive as to cause a break in the continuity of the wall of the vessel under the force of the blood pressure. Thus verrucous aortitis may lead to a spontaneous rupture of the aorta with subsequent death from hemorrhage. It may also lead to the formation of an aneurysm due to a break in the continuity of the wall of the vessel sufficiently limited to allow the other parts of the wall to become stretched and transformed into a sac. This sac may later rupture, as for instance in our case; in which the aneurysm broke into the pericardial sac. Invasion by granulation tissue from the outside may also lead to the formation of an aneurysm by disrupting and weakening the muscle and elastic fibers of the media, as was previously pointed out.

These verrucous lesions should not be confused with thrombi since they are the result of productive and regressive changes of the intima. There is no thrombus formation primarily. It is not a thromboendarteritis. In their morphology, the vascular verrucae are very similar to, if not identical with, the valvular verrucae.

The question will arise as to the specificity of the verrucous aortic lesions. In Case 1, the myocardium revealed nodular accumulations of large cells located



near the larger blood vessels. They presented the characteristic picture of Aschoff nodules. In the first case, therefore, we can definitely state that the verrucous aortitis is on a rheumatic basis. In Case 2, there were severe alterations of some of the muscle fibers of the myocardium. Between some of the muscle fibers and around the adjacent larger vessels, there were accumulations of polymorphonuclear leucocytes. Thus there was present in this case an acute myocarditis, but not specific of rheumatic fever. In this case also, there was a 2+ positive Kahn reaction on the postmortem blood, and histologically there was found evidence of a typical focal syphilitic mesaortitis. The syphilitic aortitis, however, showed no topographic or morphologic relationship to the verrucous lesion on the intima of the aorta. In the third case, there were present small scars about the blood vessels of the myocardium, which contained an occasional small accumulation of myocytes. These scars may, perhaps, have been the result of rheumatic fever, but nothing was found to substantiate this diagnosis. In Case 3 also, cocci were demonstrated. Whether these cocci have any definite relation to rheumatic fever, we are not prepared here to say. But we can, however, state that streptococci may cause lesions very similar to those observed in proved cases of rheumatic fever.

## SUMMARY

Three cases are presented which have one common feature, namely, a verrucous aortitis. In one case, which was associated with a stenosis of the isthmus, the verrucous aortitis had caused a spontaneous rupture of the aorta in an eleven-year-old child. In another, a four-year-old child, an aneurysm of the aorta developed on the basis of it and ruptured into the pericardial sac. Streptococci were demonstrated in this case.

The formation of the verrucae is explained on the basis of a fibrinoid swelling and necrosis of the ground substance of the intima with proliferation of the adjacent fibrocytes. The rupture and aneurysm are explained on the basis of necrosis destroying the internal elastic membrane with subsequent weakening of the media. The nonspecificity of the verrucous lesion of the intima of the aorta is pointed out.

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# CORYNEBACTERIUM DIPHTHERIAE GRAVIS FOUND IN MARYLAND\*

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IN A study of *C. diphtheriae* and similar organisms in Maryland, an organism resembling *C. diphtheriae gravis* described by Anderson, Happold, McLeod, and Thomson<sup>1</sup> was recently isolated. This organism was obtained from throat and nose cultures in three contact cases in a family in which there was a fatal case.

The bacteriologic findings are given in the following description.

*Colony Morphology.*—The colonies on tellurite agar in 48 hours are flatter and more spreading than those of the ordinary colonies of *C. diphtheriae*. The colonies are also dull grayish black (nonglistening) as contrasted with the glistening black colonies of the ordinary type. They have radial lines which are noticeable at 48 hours and are still more evident later. This is contrasted with the smooth colony of the ordinary type with its more pronounced concentric ridges.

*Morphology of Organism.*—Grown on Löffler's medium, the organism lacks the slenderness usually associated with the ordinary type and, when stained with methylene blue, the areas which usually stain intensely in the ordinary types take the stain less heavily, so that the general impression is that of a lighter blue. Also few or no metachromatic granules are found. When it is grown on infusion agar slants, the morphology is even more strikingly different from that of the ordinary type. The organism then is much shorter resembling *C. xerosis*. The staining is even and lighter blue, and there is an absence of granules.

*Pellicle Formation.*—In infusion broth, a pellicle forms after an incubation period of 24 hours. This is easily visible if the tubes are held up to the light and viewed from below, diagonally. There is a granular deposit, and the broth remains clear. After 48 hours' incubation, these observations are decidedly pronounced. The ordinary type shows little or no pellicle, a moderately granular formation and a definite turbidity of the broth.

*Hemolytic Action.*—There is very little hemolysis in poured blood agar plates after 24 hours' incubation as contrasted with a definite clear zone produced by the ordinary type of *C. diphtheriae*, but on further incubation, definite hemolysis is produced.

*Fermentation Reactions.*—Acid is produced from dextrose, dextrin, maltose, starch, and glycogen in nutrient broth medium. The ordinary strains do not produce acid from starch or glycogen. Acid is not produced from sucrose.

*Virulence.*—Virulence was tested intradermally by administering from 0.1 to 0.15 c.c. of suspension of a 24-hour-old growth from a Löffler slant in 5 c.c. of normal salt solution. Three-hundred-gram guinea pigs were used. The con-

\*This report represents part of a thesis study on *C. diphtheriae*. The work was done in the Bureau of Bacteriology, Maryland State Department of Health, and the Department of Bacteriology of The Johns Hopkins School of Hygiene and Public Health.

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trol guinea pig received 500 units of diphtheria antitoxin 24 hours previous to the time both control and test guinea pigs were inoculated. A very marked reaction occurred in 24 hours in the test guinea pig while little or no reaction was noted in the control animal. A zone of erythema and edema about an inch to an inch and a quarter was produced. Test guinea pigs all died in from 48 to 72 hours. Autopsy revealed primarily congested and very hemorrhagic suprarenals with considerable serous fluid in the pleural and pericardial cavities.

The differences observed in the above described organism are probably due to dissociation of the ordinary type of *C. diphtheriae*. The organism is apparently in the "rough" phase.

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### THE SEDIMENTATION REACTION IN THE NEWBORN\*

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SINCE 1918 when the sedimentation speed of the erythrocytes was first popularized by Fahraeus, there has gradually grown up a literature relating to this phenomenon, which has now reached considerable proportions. This in the main has been due to the intensive efforts of clinicians and laboratory workers to make use of this characteristic of the red blood cells as a diagnostic and prognostic agent in practically all the conditions which go to make up the field of medicine in its broadest sense. For the most part this work has been confined to adults and it is only within a relatively recent interval that the stimulus for investigative work has been transmitted over to the pediatric field.

A review of the literature quickly impresses one with the fact that a great number of methods have been evolved to obtain the sedimentation speed of erythrocytes. It is because of this variety of methods and the different terms employed in expressing the results that the reader is hard put to compare the sedimentation values as obtained by different authors and is forced to content himself with the conclusions of the author that the sedimentation reaction is normal, increased or decreased in certain conditions, pathologic or otherwise. This lack of standardization may perhaps account for the conflicting statements, as to the sedimentation reaction in normal individuals and in various diseases, appearing in the literature and undoubtedly impairs the value of the test.

Some confusion exists as to the speed of sedimentation of the erythrocytes of the newborn for Nadolny considered the sedimentation reaction to be slowest at the age of two months while György noted the contrary, claiming that

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infants over one month showed a faster sedimentation than adults whereas infants under one month showed a definite slowing in the settling of the red blood cells.

The present work was undertaken to investigate the sedimentation reaction in the newborn by the method introduced by Linzenmeir, a method which the author had employed in the study of normal and pathologic conditions in the adult. Normal infants, born of relatively normal parents, with a normal birth history were selected as the subjects of the investigation.

#### METHOD

Owing to the technical difficulty of obtaining blood from the antecubital vein of the newborn, the superior longitudinal sinus was selected as the uniform site of obtaining the blood from these infants rather than resorting to any micromethod, it being the purpose of this investigation to obtain the sedimentation reaction values in the newborn by a standard method commonly used for adults, namely, the Linzenmeir macromethod.

The infant was securely mummified on to a padded wooden board roughly corresponding to the size of the child, leaving the head as the only movable part of the infant's body. During the act of drawing blood, this part was firmly held by an assistant effectively preventing any movement of the head. The region of the anterior fontanel was made aseptically clean with iodine and alcohol, and a needle with a 2 c.c. syringe attached containing 0.4 c.c. of 5 per cent sodium citrate was inserted into the longitudinal sinus and blood drawn into the barrel of the syringe up to the 2 c.c. mark. The needle used was  $\frac{3}{4}$  of an inch long, 22 gauge and was short bevelled. The balance of the test was carried out in the usual manner of the Linzenmeir method, the time being noted when the erythrocytes reached the 18 mm. mark.

The test was carried out on infants corresponding in age to the various days of the neonatal period.

TABLE I

| NAME       | AGE IN DAYS | SEDIMENTATION TIME<br>IN HOURS |
|------------|-------------|--------------------------------|
| Baby De L. | 2           | 18                             |
| Baby R     | 3           | 14                             |
| Baby S     | 3           | 20                             |
| Baby B     | 4           | 23                             |
| Baby R     | 4           | 22                             |
| Baby C     | 4           | 16                             |
| Baby A     | 4           | 18                             |
| Baby MC    | 5           | 7                              |
| Baby N     | 5           | 17                             |
| Baby M     | 6           | 16                             |
| Baby N     | 7           | 14                             |
| Baby L     | 8           | 14                             |
| Baby K     | 9           | 12                             |
| Baby K     | 10          | 12                             |

#### COMMENT

The vena puncture of the superior sagittal sinus to obtain blood for the sedimentation tests may be open to question by some because of the possibility of damage to the substance of the brain, the formation of a blood clot, etc., but

in careful hands it is a safe method. None of the infants used as subjects in this investigation showed evidence of any trauma during the period of observation. It should, of course, be mentioned that directly following the removal of the needle pressure should be exerted over the puncture site to avoid extravasation of blood or clot formation.

With the closure of the anterior fontanel, the difficulties of obtaining blood in infants is increased unless they happen to present some veins sufficiently prominent to permit successful puncture. Therefore, it is natural that a number of micromethods should have been introduced to permit the use of small amounts of blood, not necessarily obtained from a vein, in the sedimentation test. Linzenmeir and Raunert<sup>3</sup> in 1924 reported a micromethod. One drop of blood is obtained from the lobe of the ear or tip of the finger. A capillary tube of 1 mm. width with a dilated reservoir and the upper end supplied with a thick-walled rubber tubing is filled to a certain mark with 5 per cent sodium citrate, and then the blood is aspirated to another mark so that a ratio of 1:4 is obtained. The blood is mixed in the dilated part of the tube and is placed in a rack and sealed with a rubber plate. The sedimentation time is read as in the macromethod.

Numerous other micromethods have been reported in the literature since then by Langer and Schmidt,<sup>4</sup> Cutler,<sup>7</sup> Poindecker,<sup>6</sup> Haselhorst,<sup>5</sup> Kowarski,<sup>8</sup> Ackerman,<sup>9</sup> and others, but all of them depend upon the securing of blood by means of some type of capillary tube. It is generally felt that the micromethod, although slightly less reliable than the macromethod, nevertheless gives sufficiently good clinical results to be of value especially where blood cannot be easily obtained from a vein, as often happens in infants and obese individuals. Investigation has shown that a capillary tube less than 1 mm. in diameter leads to irregular results. Kowarski also calls attention to the fact that blood from the tip of the finger settles somewhat faster than venous blood. This difference can be compensated for by changing the quantitative proportion of citrate solution to the blood, thus, Kowarski uses a dilution of 1:2.5.

#### DISCUSSION

According to the results obtained in this investigation, the sedimentation time in the normal newborn infant seems to range between seven and twenty-three hours, with the average sedimentation speed for the entire neonatal period being about fifteen hours as compared to the two hours considered to be normal in adults. There is also reflected a tendency for the sedimentation reaction to become less prolonged as the infant grows older.

Silzer<sup>11</sup> in a study of the sedimentation speed of red blood cells from the umbilical vein of 800 infants noted that 91.75 per cent of the cases showed a sedimentation time over twenty-four hours; 4.25 per cent showed sedimentation in from twelve to twenty-four hours; 3.8 per cent in from one to twelve hours and  $\frac{1}{2}$  per cent showed sedimentation in from thirty to sixty minutes. That the blood fibrinogen is probably the most important factor in the prolongation of the sedimentation reaction in infants is suggested by the work of Bruchsaler<sup>12</sup> who studied the relative sedimentation reaction and blood fibrino-

gen in infants and pregnant women and found that the maternal blood showed from 250 to 500 units of fibrinogen while the blood of the newborn infants contained from 64 to 125 units.

#### CONCLUSIONS

1. The speed of sedimentation of the erythrocytes in the newborn is considerably prolonged.
2. There is a tendency for the sedimentation reaction to be less retarded as the infant becomes older.
3. The use of the superior longitudinal sinus as a source of obtaining blood for the study of the sedimentation speed in the newborn is discussed.

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## OBSERVATIONS ON THE VASCULAR RESPONSE TO DRAINAGE OF ASCITES\*

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IT HAS been observed that venous pressure may fall after excessive abdominal distention has been released. Plumier,<sup>1</sup> in a series of animal experiments, found a drop in the femoral vein pressure after release of fluid previously injected into the peritoneal cavity. Villaret and Saint-Girons<sup>2</sup> noted a temporary fall in venous pressure after ascites was removed from patients with portal cirrhosis. Brams, Katz, and Kohn<sup>3</sup> noted a marked fall in pressure in the femoral vein in animal experiments after abrupt release of abdominal distention. On the other hand, the pressure in the external jugular changed in only a few instances, the fall being much less than in the femoral vein. The carotid pressure in these experiments varied on abrupt release of abdominal distention; a fall, either transient or prolonged, occurred in about two-thirds of the experiments, and a rise was present in the remainder.

The present research was undertaken (a) to study the circulatory responses *during* the removal of ascitic fluid in patients with portal cirrhosis and chronic ascites, and (b) to compare the results of such gradual release of abdominal distention in these patients with the effects of more abrupt release in animal experiments.

The direct method of determining venous pressure was used. A hollow needle was inserted into the median basilic vein and connected with a manometer by suitable rubber tubing. The entire system was filled with 3 per cent sterile citrate solution from a reservoir. A little citrate solution was permitted to flow into the vein through the needle every few minutes in order to prevent clotting. Compression of the veins of the arm was prevented by adducting the extremity. Patency of the needle was tested before and after each reading of venous pressure. Sufficient time was allowed in all instances for stabilization of the level of the citrate solution in the manometer. The level of the right auricle was marked in the fourth right interspace at the junction of the middle and anterior thirds of the thorax and this point was used as the zero level on the manometer. This level was determined before each reading in order to prevent errors due to change in position of the patient during the course of the drainage.

Ten patients with chronic ascites of marked degree due to portal cirrhosis were selected for this study. Three of the patients were in the recumbent position and 7 were sitting during the drainage. Paracentesis was performed in the hypogastrium with a large trocar under aseptic precautions and after a preliminary local anesthesia. A long rubber tube was attached to the trocar in

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order to siphon the ascitic fluid into a receptacle resting on the floor. The fluid was removed in stages, a liter at each stage in 8 experiments and in larger quantities in the other two. Venous pressure, systolic and diastolic arterial pressure, and pulse rate were noted at each stage. Respirations and the general condition of the patient were also closely observed in all instances.

The control venous pressure was normal or slightly elevated in 9 of the patients and low in one. This does not agree with Villaret and Saint-Girons<sup>2</sup> who reported a low venous pressure in all their patients with portal cirrhosis.

TABLE I

PROTOCOL OF CASE 6, TYPICAL OF THOSE OBSERVED IN ALL INSTANCES (FEMALE, AGE FIFTY YEARS; DIAGNOSIS, PORTAL CIRRHOSIS OF LIVER WITH CHRONIC ASCITES; SITTING POSTURE IN BED)

| AMOUNT FLUID<br>REMOVED<br>(C.C.) | VENOUS PRESSURE<br>MM. CITRATE | BLOOD<br>PRESSURE<br>MM. HG | PULSE<br>RATE PER<br>MINUTE |
|-----------------------------------|--------------------------------|-----------------------------|-----------------------------|
| Control                           | 108                            | 142/128                     | 132                         |
| 1000*                             | 105                            | 144/128                     | 108                         |
| 2000                              | 92                             | 144/128                     | 116                         |
| 3000                              | 95                             | 142/128                     | 120                         |
| 4000                              | 80                             | 142/118                     | 120                         |
| 5000                              | 70                             | 136/112                     | 126                         |
| 6000                              | 85                             | 134/108                     | 128                         |
| 7000                              | 72                             | 136/106                     | 116                         |
| 8000                              | 70                             | 134/104                     | 120                         |
| 9000                              | 60                             | 132/100                     | 116                         |
| 10000                             | 60                             | 130/104                     | 124                         |
| 11000                             | 58                             | 129/102                     | 120                         |
| 12000                             | 58                             | 129/102                     | 120                         |
| 13000                             | 65                             | 128/98                      | 108                         |
| 14000                             | 60                             | 124/98                      | 106                         |
| 15000                             | 50                             | 132/108                     | 124                         |
| 16000                             | 55                             | 128/106                     | 116                         |
| 17000                             | 50                             | 128/98                      | 122                         |
| 18000                             | 40                             | 120/96                      | 120                         |

\*Five minutes elapsed between removal of each 1000 c.c.

The changes in Case 6 are typical of those which occurred in all instances and are shown in Table I. An early fall in venous pressure occurred in all 8 patients in whom determinations were made after the removal of each 1,000 c.c. of ascitic fluid. In four of these the lower level of venous pressure was maintained, while in the remaining 4, a further fall occurred as more fluid was withdrawn. In all of the patients the venous pressure was lower at the end of the drainage than at the beginning.

The systolic arterial blood pressure fell in all but one of the patients, the drop varying from 4 to 34 mm. of mercury. This is in contrast to the variable results noted after abrupt release of abdominal distention in animals.<sup>3</sup> The diastolic blood pressure fell in all instances, the decrease varying from 4 to 32 mm. of mercury. The arterial blood pressure, like the venous pressure, began to fall early in the experiment. Faintness occurred in one instance where the systolic blood pressure fell from 94 to 60 mm. of mercury and the diastolic, from 76 to 54. The venous pressure in his patient had been falling gradually from the beginning, from 80 to 54 mm. of citrate solution. At the

time the patient complained of vertigo, the venous pressure fell abruptly from 54 to 35 mm. of citrate solution. The patient recovered in a few minutes after which the systolic blood pressure rose to 74 and the diastolic to 54 mm. of mercury. The venous pressure also rose at this point to 43 mm. of citrate solution. The fall in arterial pressure in this patient occurred before the venous pressure dropped; a finding in accord with that reported by Brams, Katz and Kohn<sup>3</sup> in animal experiments.

The pulse rate was slower in 6 patients at the end of the experiment and unchanged in 4. It is interesting to note that no increase in pulse rate occurred during the period of faintness in the patient described above in detail.

The early fall in venous pressure appears to be due chiefly to improvement in respiratory excursion. The respirations became definitely deeper after some ascitic fluid was removed. A further fall in venous pressure occurred in these patients when they were instructed to breathe more deeply. A similar fall in venous pressure was reported by Meyer and Middleton<sup>4</sup> during the hyperpneic stage of Cheyne-Stokes respiration and by Kroetz<sup>5</sup> after continuous deep breathing. The mechanism for the venous pressure drop might be the increased aspiration of blood into the thorax accompanying the greater respiratory excursion.

The fall in arterial blood pressure and pulse rate is more difficult to explain. A number of factors were pointed out in the discussion of the results obtained in animal experiments; the most likely factors being (1) decrease in peripheral resistance due to reduction of compression of the abdominal aorta, arterioles, and capillaries and (2) reflexes via the splanchnic nerves.

The clinical significance of a fall in blood pressure is important, as a marked drop such as occurred in some of our patients may lead to collapse. In a patient with a normal blood pressure and a quickly adjusting vasomotor reaction, no harm may result but even a small blood pressure drop may be serious if the blood pressure is low to begin with. A subsequent study of all 10 cases showed that the only patient in whom collapse occurred was in an instance where the blood pressure before tapping was 94/78. Further observations are necessary to determine whether such a low blood pressure warrants caution in the removal of ascites. Another patient (Case 3) went into collapse on two preceding occasions when the fluid was removed while in a sitting posture. His systolic blood pressure was 142 mm. of mercury and the diastolic was 90. Removal of the same quantity of ascitic fluid at the same rate as on the previous occasions resulted in no untoward effects when drainage was performed in the supine position. It is possible that this patient with a normal blood pressure is an example of failure of the vasomotor system to adapt itself sufficiently to the changes induced by removal of ascites. In any event, it is suggested that the patient be placed in a recumbent position in all cases where ascites is to be drained, and a hypodermic syringe of adrenalin be available.

#### SUMMARY

1. The effect of draining off abdominal fluid on the venous pressure, systolic and diastolic arterial blood pressure and pulse rate was studied in 10 patients with portal cirrhosis and chronic ascites.

2. The venous pressure began to fall early during the drainage, and in four instances continued as more fluid was removed. The venous pressure at the end of the drainage was lower in every instance than before tapping was begun.

3. Systolic and diastolic arterial pressures fell while the fluid was being removed. A fall in blood pressure was associated with faintness in one patient whose pressure was low before the procedure was begun. A similar fall in other cases with previously normal blood pressure was not associated with such untoward symptoms.

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## PIGMENT STUDIES<sup>2</sup>

### I. ON A BROWN SKIN-ADRENALIN COLOR REACTION

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MEIROWSKY<sup>1</sup> seems to have been first to call attention to the fact that a melanin-like substance forms itself when an extract of human skin is heated in the presence of adrenalin. His original experiment was made with a mixture that consisted of "suprarenin (1:1,000)" one part, extract of preputial skin one part, and water one part. After this preparation had been incubated for twenty-four hours at 56° C., he noted that its color had changed from a light yellow to an intense grayish black. More recently<sup>2</sup> it has been shown that sections of whole skin exhibit a like reaction.

Two questions about this striking phenomenon still await elucidation—one concerns the chemical composition of the pigment substance itself and the other, its mode of formation.

With respect to the second query—the only one considered here—the view seems to prevail very generally that the pigment is produced in some way through the activity of an oxidizing ferment present only in living epithelial cells. Indeed, having shown experimentally that sections of human skin treated with a 1 to 2 per cent aqueous solution of 3, 4-dioxyphenylalanin exhibit a similar color change. Bloch<sup>3</sup> insists that the brownish substance is formed through the agency of a specific intracellular ferment, the so-called

<sup>2</sup>Received for publication, August 30, 1933.

dopa oxydase. This ferment is said to be thermolabile<sup>3</sup> and to be rendered inactive, if not actually destroyed, at a temperature of 57° C.

The work herein reported was undertaken in order to ascertain, in regard to the factor of temperature whether sections of human skin would display a peculiarity previously noted in experiments with a finely powdered preparation of ricinus lipase. Concerning this peculiarity, it was found constantly on incubating a small quantity of the lipase material in a dilute solution of adrenalin at 45° C. that in the course of a week or two the color of the precipitated powder gradually changed from white to brownish black. But, on the other hand, it was also noted constantly that this color reaction took place with equal, if not slighter, intensity when such experiments were repeated with a lipase powder previously sterilized by heating it in steam for half an hour at 100° C. To check this finding by means of another tissue, similar experiments were made with bits of human skin, using as test objects small squares of it that measured about 5 mm. on a side.

#### MATERIAL AND TECHNIC

The working material used was obtained, as needed, from 26 selected seemingly normal, coroner's subjects. Qualitative details about this material may be dispensed with, as the various specimens of skin tested did not seem to differ from one another significantly as regards their pigment-forming capabilities. It should be stated, however, that care was taken to select skin that was both white in color and free from coarse hairs. Each specimen was prepared for use by paring away as much as possible of the subcutaneous fat. This having been done, and the skin having been washed clean in running tap water and dried with a cloth, it was then cut into squares of the proper size.

It was found convenient to make the experiments in small test tubes (1 cm. in diameter). The preparation used routinely consisted of a single test square and 4 c.c. of an adrenalin solution made by diluting Parke, Davis and Company's adrenalin solution (1:1,000) with freshly boiled distilled water in the proportion of 1 to 5. Whether fresh or steamed, the squares were tested in three ways, namely, in an open tube, in another sealed by a layer of heavy mineral oil and in a third tube sealed by fusing the open end over a Bunsen burner. Toluene was added only to the unsealed preparations. The tests were set up in parallel series. In one of these the skin was tested in its original, fresh state, in the other, after it had been heated for half an hour in moist

TABLE I\*  
PIGMENT FORMATION IN HYDROLYZING SKIN-ADRENALIN SYSTEMS

| MATERIAL TESTED         | PREPARATIONS |                        |                        |
|-------------------------|--------------|------------------------|------------------------|
|                         | OPEN         | SEALED<br>UNDER<br>OIL | SEALED<br>BY<br>FUSION |
| Squares of fresh skin   | 73           | 60                     | 104                    |
| Squares of steamed skin | 58           | 58                     | 67                     |

\*Shows average time interval, in hours, before appearance of a brown substance in preparations made, respectively, with squares of living skin and squares of devitalized skin. Values shown are based on data noted in parallel series of experiments with skin specimens obtained from 26 coroner's subjects. Incubation temperature 45° C.

steam at 100° C. Satisfactory results were obtained by incubating the preparations at a constant temperature of 45° C.

#### RESULTS

When such preparations were placed in the incubator and observed from day to day, in the course of from twenty-four to seventy-two hours it was noted that the adrenalin solution became faintly pink in color; and that immediately after this coloring matter made its appearance—never before—the skin squares began to turn brown. This change was at first barely perceptible as an extremely pale shade of brown. But the reaction being a progressive one, often in the course of from six to ten days, the squares became almost black in color. There seemed to be a relationship of some sort between the rate of formation of the brown pigment and the color of the supernatant adrenalin solution. For it was obvious that the brown material was produced but slowly while the adrenalin solution was pale pink in color, at a more rapid rate while it exhibited a clear, rose pink shade, and most rapidly when its color had become reddish brown. These color changes occurred earlier and seemed to be somewhat more pronounced in the preparations made with squares of devitalized skin than in those made with squares of living skin; the average values obtained as regards the time of onset of the brown staining effect were, respectively, fifty-eight hours and seventy-three hours (Table I). Formation of the brown dyestuff took place in both of the preparations from which air was excluded. But on the whole it formed more slowly in the preparations sealed by fusion than in those sealed by a layer of mineral oil.

In numerous collateral series of experiments it was noted, constantly, that the addition of 1 per cent by weight of sodium chloride to a preparation caused the square—whether a heated or an unheated one—to turn brown within twenty-four hours and to exhibit a maximum grade of pigmentation within a period of four days.

Control squares of skin were incubated in distilled water at 45° C. Without exception, after an incubation period of two weeks each square remained as perfectly white as it had been when in the fresh state.

#### SUMMARY

1. Experimental data are presented which show that bits of human skin become dark brown in color on being incubated in a dilute solution of adrenalin.
2. The results obtained seem to show conclusively that the brown pigment forms itself in the absence of any organized ferment.

The author wishes to thank Mr. Paul C. Greene for valuable technical assistance rendered during the course of this study.

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## PIGMENT STUDIES\*

### II. ON THE IMPORTANCE OF HYDROLYZED ADRENALIN AS A TISSUE STAIN

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IN THE preceding study it was found with respect to two organic substances, one of vegetable, the other of animal origin, that they gradually became almost black in color if they were incubated for some time at 45° C. in a hydrolyzing solution of adrenalin; and that this phenomenon occurred whether these substances were tested in the fresh state or after they had been heated in live steam for half an hour at 100° C. The materials tested were small quantities of defatted castor bean meal and bits of human skin.

Apparently, the conditions under which the experiments had been made were such as to preclude the possibility that the melanin-like pigment had formed itself through the agency of an organized ferment. It seemed probable, nevertheless, that the process of pigment formation was essentially a catalytic one; and that the color change noted took place because of the fact that the solution contained some cleavage product of adrenalin which acted upon the tissues and dyed them brown. In other words, the evidence seemed to indicate that the solution of hydrolyzed adrenalin acted simply as a non-specific stain. As it seemed worth while to secure, if possible, some data on this point, many series of experiments were made with suitable test objects representative of various cellular structures. The results obtained show that under proper conditions a hydrolyzing solution of adrenalin is capable of imparting an intense brownish black color to a number of organic substances. Since it does not appear to be generally known that such a solution has tinctorial properties, this paper gives a brief account of the staining experiments.

#### MATERIAL

Four kinds of material were tested: white holly, normal human skin, normal human red bone marrow, and blood obtained from a patient afflicted with myelogenous leucemia.

The white holly was purchased from a cabinetmaker. It consisted of small sheets of veneering, about 1 mm. in thickness and perfectly "dressed" on both surfaces. Pieces of this wood 1 cm. square were used in the experiments. The specimens of skin tested, as well as those of bone marrow, were obtained, as needed, from carefully selected coroner's subjects. The tissues were prepared for use as follows: Each skin specimen, in the usual manner, was first hardened in 10 per cent formaldehyde and then embedded in paraffin and sectioned. The staining tests were made with sections attached to glass slides by means of an albumin fixative. In the experiments made, respectively, with red bone marrow and leucemic blood, the usual smear preparations were used. Both were fixed in absolute alcohol.

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## TECHNIC

1. *Preparation of the Staining Solution.*—Into a cylindric specimen tube 3 cm. by 8 cm. in size, 3 c.c. of Parke, Davis and Company's adrenalin solution (1:1,000) and 7 c.c. of freshly boiled distilled water were measured by means of a pipette and then, to serve as a catalytic agent, a bit of human skin 5 mm. square—previously steamed for half an hour at  $100^{\circ}$  C.—was dropped into the tube. To prevent loss by evaporation the tube was provided with a tightly fitting stopper. The preparation then was incubated at  $45^{\circ}$  C. and observed from day to day with reference to the appearance of two color reactions—one in the supernatant solution, the other in the little piece of tissue. As a rule, in the course of from one to three days, the solution gradually became deep rose pink in color while, toward the end of that period, the bit of skin began to exhibit a very pale brownish discoloration. When both of these color changes were visible, the solution was ready for use.

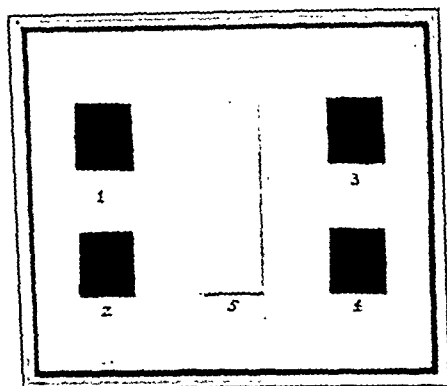


Fig. 1.—Squares of white holly stained in a solution of hydrolyzing adrenalin. One and three are squares of the wood in its natural, unheated state. Two and four are squares steamed for half an hour at  $100^{\circ}$  C. before they were tested. Five is the control strip. Incubation period was six days. Temperature  $45^{\circ}$  C. Note that the heated squares are darker in color than the unheated ones.

2. *The Staining Procedure.*—Slide preparations of the tissues to be tested were brought into the rose pink solution and then incubated at  $45^{\circ}$  C. until the desired brown staining effect developed. This usually required a period of from six to ten days. It should be mentioned that the squares of white holly were introduced into the solution edgewise and kept in a vertical position by means of suitable holder devices fashioned out of glass tubing.

## RESULTS

*White Holly*

Experimental evidence was sought especially in regard to two points: (1) Does heat modify the action of the stain in any way? (2) Is the staining process modified by the factor of concentration?

*Influence of the Heat Factor.*—Method: Two pieces of white holly were used in each experiment, namely, a square of the wood in its natural, unheated state, and a square that had been steamed for half an hour at  $100^{\circ}$  C.

Results: On removing the squares from the solution after the proper time interval, it was found constantly (1) that both of the squares had changed in color from white to brown; (2) that the heated square was several shades darker in color than the unheated one. (See Fig. 1.)

*Influence of the Concentration Factor.*—Method: Proceeding in the manner already described and using in each instance a bit of steamed skin as the catalyst, a series of five staining solutions, regularly graded in concentration according to the amount of adrenalin present in each one, were made ready for use in the small cylindric containers. By volume these preparations contained, respectively, 20, 40, 60, 80, and 100 per cent of commercial adrenalin solution (1:1,000). Having then placed in each container a square of unheated white holly to serve as a test object, the preparations were incubated at 45° C. for the usual length of time.

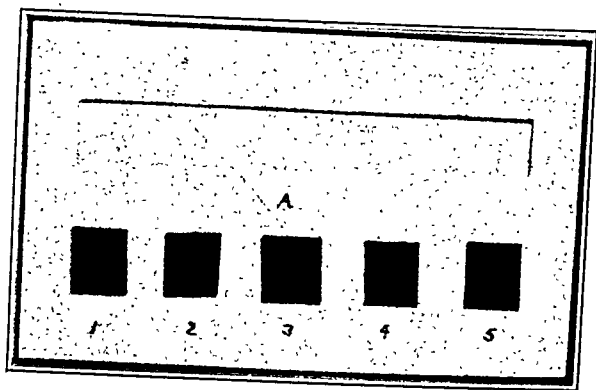


Fig. 2.—Showing influence of the concentration factor. The unheated squares, one to five were stained simultaneously in solutions that contained, respectively, 20, 40, 60, 80, and 100 per cent of adrenalin (1:1000). Incubation period six days. Temperature 45° C.

Results: In a typical experiment, after the lapse of six days it was found that the set of squares displayed the color changes of graded intensity shown in Fig. 2. The tone range was from pale brown to brownish black. The differences between individual squares of a series, as regards the relative depth of the staining effect, were rendered remarkable by a particular circumstance; for it will be noted that the grain of the wood stands out more or less conspicuously in the first three squares, stained in the weaker solutions, while the remaining two squares appear to be uniformly black in color.

### Skin

Hope was entertained that suitable tests with thin sections of skin would serve to explain a peculiarity in regard to the distribution of the brown dye-stuff previously noted in the experiments with whole skin. For on examining bits of blackened skin removed from old testing solutions, it had been observed invariably that the brownish black staining effect was most pronounced at or near the free epithelial surface of the specimen while, in striking contrast, the deeper, subcutaneous tissues showed much lighter degrees of discoloration. Since it seemed probable that thin sections of skin would stain more



evenly, numerous slide preparations were incubated in the staining solution until the proper color developed and then were examined under the microscope. Contrary to expectation, however, it was found in every case: (1) That the amount of the brown material present in the tissue increased in the direction from the depth toward the free surface of the section. (2) That the deep layers of the stratum corneum were so intensely stained that an average section (see Fig. 3) had the appearance of being bordered on one side by a sharply defined zone wherein the black staining effect closely resembled that observed in the experiments with white holly.

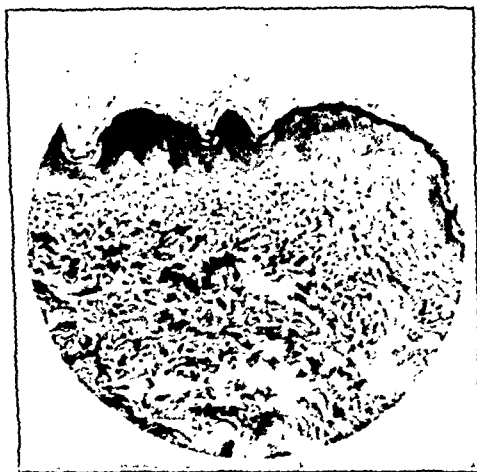


Fig. 3.—Section of normal human skin stained in hydrolyzed adrenalin for six days. ( $\times 125$ )

Comment: The foregoing observations seem to justify the inference that the depth of the brown discoloration at any given level in a stained section of skin is related in some way to a density factor. At any rate, figuratively speaking, it would appear that somewhat as the lines in a contour map indicate the pitch of a slope by their nearness to one another, so in a stained section of skin the color appears to be darkest near the surface where the cells are closely packed.

#### *Leucemic Blood and Red Bone Marrow*

Method: As it was desired to find out what staining effects, if any, would take place if representative groups of leucocytes were incubated in the adrenalin solution, simultaneous tests were made with two smear preparations—one of leucemic blood and one of bone marrow. Twenty experiments were made in all. The average time period was six days.

Results: Although the pigmentary changes observed varied considerably both in degree and extent, it was found, without a single exception, that the leucocytes exhibited brown color reactions. In a majority of the specimens the staining effects were comparatively slight in degree and were limited to the nuclei, causing these to appear as pale brown objects sharply outlined within the colorless cell bodies. But not a few other specimens were encoun-

tered (see Figs. 4 and 5), which afforded obvious proof that the brown dye had stained deeply not only the nuclei of the leucocytes but also the myelocytic granules. Therefore, having in mind the evidence previously acquired, it was inferred from these observations, (1) that the test solution contained a diffusible nonspecific pigment of unknown composition; and (2) that al-

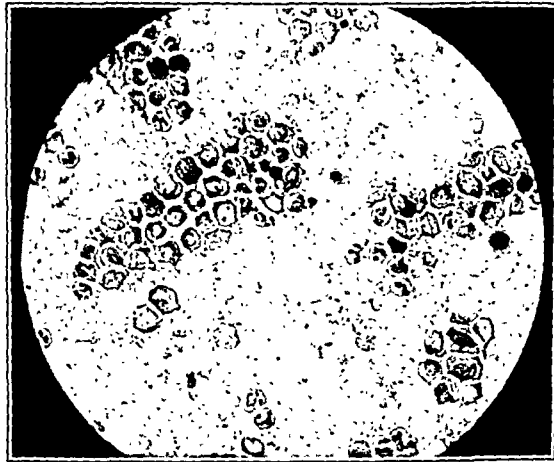


Fig. 4.—Smear of leucemic blood stained in hydrolyzed adrenalin for six days.

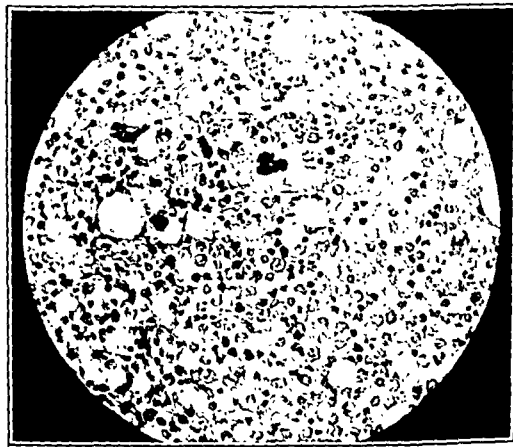


Fig. 5.—Smear of human red bone marrow stained in hydrolyzed adrenalin for six days.

though the conditions essential to the development of its maximum activity were undetermined, this substance stained the component tissues of the leucocytes with unequal intensity.

#### SUMMARY

1. A new method is presented for the preparation of a rose pink solution of hydrolyzed adrenalin.
2. Under suitable conditions this solution imparts a deep brown or brownish black color to various organic substances.

3. The brown dye appears to be nonspecific in its action.
4. The staining effects obtained with specimens of white holly, human skin, leucemic blood and red bone marrow are described.

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## CRYSTALLINE ELEMENTS IN THE STOMACH LAVAGE OF PATIENTS WITH CHOLELITHIASIS\*

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IN A PREVIOUS paper,<sup>1</sup> certain types of crystals, present in preoperative specimens of bile, in patients with gallbladder disease, were described and compared with biliary crystals found in the scrapings of stones and bile removed from the gallbladder at operation. During the course of the above investigation, it was observed that in some patients, the duodenal tube did not pass beyond the pylorus within a period of eighteen hours. A microscopic examination of the aspirated gastric contents of these patients revealed various types of biliary crystals. It was, therefore, thought of interest to determine the frequency with which crystalline elements were found in the gastric contents of patients with gallbladder disease. For this study, 28 patients were observed.

The patients were grouped as follows: Group 1, consisted of 21 patients, in whom there was definite roentgenographic evidence of cholelithiasis. Group 2 (the control group), consisted of 7 patients who were free from gastrointestinal symptoms.

The gastric contents of all the patients were obtained by stomach lavage, because (lavage) water was found to be the best medium for the microscopic detection of biliary crystals. The gastric lavage was performed with plain tap water, preferably in the morning, on any empty stomach. A specimen of the lavage water was then collected and examined microscopically.

In Group 1, there were 21 patients. Five of these were males, and 16 females, the ages ranging from thirty-one to sixty years. Typical and atypical cholesterol crystals, carbonate crystals, and calcium bilirubinate pigment (granular and amorphous types), separately or collectively, were found in the gastric lavage of every one of the patients in this group. The crystals and pigment were present in appreciable amounts, and as follows: Cholesterol and calcium bilirubinate pigment, together in 14 patients; cholesterol and carbonate crystals together with calcium bilirubinate pigment in 3 patients (Figs. 1 and 2); cholesterol crystals alone in 2 patients; and calcium bilirubinate pigment alone in 2 patients. The cholesterol type of crystal predominated in 9 of the patients in this group. Calcium bilirubinate pigment was most abundant in

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12 patients. The crystalline elements were found, at times, in the mucus, and, at other times, independent of the mucus. It was not necessary to centrifuge the specimens to obtain the crystals.

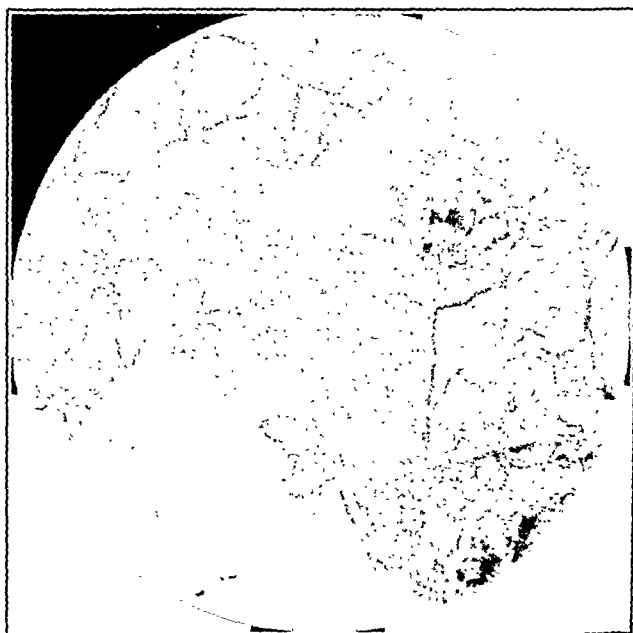


Fig. 1.—Mrs. K. Cholesterol and carbonate crystals; slight amount of calcium bilirubinate pigment. (Microphotograph of particle in preoperative specimen of gastric lavage.)



Fig. 2.—Mrs. F. Abundant amount of calcium bilirubinate pigment and few cholesterol and carbonate crystals. (Microphotograph of particle in preoperative specimen of gastric lavage.)

Eighteen of the patients in Group 1 were operated upon. In all of these cases, the crystalline elements observed in the lavage water, preoperatively, were found to be similar in appearance to the biliary crystals seen in the scrapings of stones or bile removed from the gallbladder at the time of operation.

Group 2 (the control group) consisted of 7 patients ranging in age from twenty-five to fifty years, all of whom were free from gastrointestinal symptoms. Four of these patients were males, and 3 were females. These patients came to the hospital for other than abdominal ailments, and, as far as could be ascertained, never complained of gastric symptoms. Microscopic examination of the stomach lavage obtained from these patients did not reveal the presence of cholesterol or carbonate crystals. In only 4 of the patients a slight amount of calcium bilirubinate pigment was found.

# COMMENTS

Cholesterol and carbonate crystals and calcium bilirubinate pigment were found, separately and collectively, in appreciable amounts, in the stomach lavage of a group of patients suffering from calculous cholecystitis. The presence of these crystalline elements in the gastric lavage was due to regurgitation of the bile and duodenal contents into the stomach. In about one-half of the patients the gastric lavage water was bile tinged, and, in the remaining patients, it was colorless.

Although biliary crystals were present in the stomach lavage in appreciable amounts, they were not, as a rule, as numerous as the crystals seen in preoperative specimens of bile of patients with cholelithiasis. When, however, the gastric lavage was performed within six hours after the onset of an attack of biliary colic, showers of crystals and an abundant amount of calcium bilirubinate pigment were found.

Carbonate crystals in the stomach lavage were dissolved by hydrochloric acid which aided in differentiating these crystals from the atypical types of cholesterol crystals. At times, starch granules in the lavage water had to be distinguished from biliary crystals. This was readily done by the addition of iodine.

Microscopic examination of the stomach lavage of the patients in the control group did not reveal any cholesterol or carbonate crystals; yet, a slight amount of calcium bilirubinate pigment was found in four of the patients. In a previous paper,<sup>1</sup> it was emphasized that a few cholesterol crystals or a small amount of calcium bilirubinate pigment, present in preoperative specimens of bile, had no pathologic significance. The presence of a slight amount of calcium bilirubinate pigment in the stomach lavage, likewise, cannot be regarded as abnormal.

The presence of biliary crystals in the stomach lavage of patients with cholelithiasis, is of some significance. However, an exact diagnostic appraisal of this finding cannot as yet be given, as this subject is still under investigation.

# CONCLUSIONS

1. A series of 21 patients with cholelithiasis, and another 7 patients (control group) free from gastrointestinal symptoms, were subjected to a microscopic examination of the gastric contents obtained by lavage.

2. The stomach lavage of the patients with calculous cholecystitis showed appreciable amounts of cholesterol and carbonate crystals and calcium bilirubinate pigment occurring separately and collectively.

3. In the control group the gastric lavage showed, in only four patients, a slight amount of calcium bilirubinate pigment, which is not regarded as abnormal.

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## EFFECT OF SMOKING ON SKIN TEMPERATURE\*

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CONSIDERABLE investigation has been carried out during the past decade in an attempt to determine the various effects of tobacco on the human body. It has long been thought that tobacco was an important contributing etiologic factor in some of the vascular diseases, most notably thromboangiitis obliterans. Certain other conditions also have been so frequently associated with the practice of smoking that the causal connection has been considered indisputable by some authorities. In this group we can mention cardiac arrhythmias, shortness of breath, and chronic congestion of the nasopharynx.

On chemical analysis of tobacco smoke, Bogen<sup>1</sup> points out that the composition of the smoke obtained from any cigaret depends to a great extent on the manner and rate with which the cigaret is smoked. The sidestream, or the smoke from the lighted end of the cigaret, ordinarily constitutes the greater part of the actual materials given off from the combustion. This contains a low concentration of carbon monoxide with a relatively high percentage of nicotine. Smoke which has been sucked in contains an appreciable amount of carbon monoxide. This has been estimated as high as 8.3 c.c. from each gram of tobacco smoked, which is approximately the amount of tobacco in each cigaret. The amount of nicotine in the smoke sucked in is much greater than that of the sidestream. This has been estimated at from 3 mg. to 9 mg. from each gram of tobacco smoked, and 88 per cent of this is absorbed. Bogen notes also that domestic cigarets are somewhat richer in nicotine than many of the imported brands. The domestic brands average about 2.5 per cent nicotine.

Whether or not nicotine is the primary toxic agent in tobacco smoke, it is interesting to note that according to Bastedo<sup>2</sup> nicotine is rapidly absorbed from skin and mucous membranes. Its main action is a brief stimulation of the sympathetic and parasympathetic ganglia, followed by a depression. The stimulation of the ganglia results in a rise in blood pressure. The lethal dose of nicotine for an adult is about 500 mg. In addition to the nicotine and carbon monoxide, the aspirated smoke contains pyridine, aldehydes (especially furfural), and volatile oils. These are generally considered of relatively little significance.

That smoking has an effect on the cardiovascular system has long been

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suspected. Simici and Marco<sup>1</sup> in 1926 stated that tobacco smoke produces in both smokers and nonsmokers well-marked disturbances in the cardiovascular system. These disturbances, they consider, are characterized principally by phenomena of vasoconstriction which appear first in the visceral, and then in the peripheral vessels. They found a rise in blood pressure of 20 to 30 mm. of mercury, with an acceleration of pulse of 10 to 40 per minute. They explain this on the basis of a vasoconstriction.

Bruce Miller and Hooker<sup>4</sup> in 1909 believed that there was probably a peripheral vasoconstricting effect from smoking tobacco and demonstrated this by noting the volume changes in the arm and hand. Moschcowitz<sup>2</sup> in 1928 stated that the most probable explanation of the so-called tobacco angina is that it is due to the constrictive action of tobacco on the blood vessels of the heart.

Madoch and Coller<sup>5</sup> have more recently shown what they consider further evidence of a peripheral vasoconstriction as a result of smoking, by determining the temperature of the skin of the upper and lower extremities. In the case mentioned in their report, they found a drop of  $5.9^{\circ}$  C. in the upper extremity and  $1.5^{\circ}$  C. drop in the lower extremity while the subject smoked 3 cigars.

In the present study 34 subjects were used. An attempt was made to obtain a record of the changes in the temperature of the skin while the subjects were smoking. The region just below the nail of the third finger of the left hand was selected as the site for temperature determination. The subject's hand was placed in a comfortable position and relaxed. The instrument used for measurement was the Leeds and Northrup electric recorder. The thermocouple was fastened to the site selected and the record taken.

After a constant temperature was obtained the subject was instructed to smoke a cigaret in his accustomed manner, continuing to smoke until approximately  $\frac{3}{4}$  of the cigaret had been consumed. Several of the popular brands were used. Room temperature varied between  $63^{\circ}$  F. and  $86^{\circ}$  F. In no case did the lowest temperature reached go below room temperature during the progress of the experiment.

In Table I 26 of the subjects were habitual smokers, consuming from 15 to 30 cigarettes daily. Of these, 22 showed a drop in skin temperature averaging  $4.3^{\circ}$  F., 2 showed no change at all, and one showed an elevation of  $8^{\circ}$  F.

Four of the subjects in Table II were retested, and in each case a comparable change was noted except one, where the drop was approximately half of the original.

In the group of 8 nonsmokers, 3 showed an average drop of  $3.2^{\circ}$  F., 4 showed no change at all, and 1 showed an elevation of  $1^{\circ}$  F.

Four cases of thromboangiitis obliterans were studied, 3 of which showed an average drop of  $8^{\circ}$  F. One showed no change at all.

In practically all of the cases studied where a drop in temperature was noted, the temperature change was evident before one-half the cigaret had been consumed. The average time required to reach the lowest temperature from the beginning of smoking was fourteen minutes, with five minutes as the minimum and fifty minutes as the maximum. The time required to return to the initial temperature or higher after the low point, averaged twelve minutes with five as the minimum and twenty-five as the maximum.

TABLE I  
HABITUAL SMOKERS

| SUBJECT | ROOM TEMP. | INITIAL TEMP. | LOWEST TEMP. | DEGREES DROP | TIME OF DROP | RETURN TEMP. | TIME OF RETURN | USUAL DAILY CONSUMPTION OF CIGARETS |
|---------|------------|---------------|--------------|--------------|--------------|--------------|----------------|-------------------------------------|
| 1       | 63         | 75.0          | 72.5         | 2.5          | 12           | 91.0         | 14             | 20                                  |
| 2       | 63         | 85.0          | 80.6         | 4.4          | 8            | 86.0         | 5              | 20                                  |
| 3       | 72         | 79.0          | 76.0         | 3.0          | 10           | 83.0         | 20             | 15                                  |
| 4       | 72         | 81.0          | 78.0         | 3.0          | 12           | 81.0         | 6              | 15                                  |
| 5       | 68         | 71.0          | 67.6         | 3.4          | 5            | 70.0         | 15             | 30                                  |
| 6       | 68         | 94.4          | 92.6         | 1.8          | 5            | 94.5         | 8              | 30                                  |
| 7       | 70         | 87.0          | 76.0         | 11.0         | 20           | 78.0         | 5              | 15                                  |
| 8       | 72         | 93.0          | 88.0         | 5.0          | 8            | 92.0         | 10             | 15                                  |
| 10      | 68         | 86.0          | 79.5         | 6.5          | 12           | 87.0         | 8              | 15                                  |
| 11      | 68         | 87.0          | 81.5         | 5.5          | 17           | 89.0         | 14             | 15                                  |
| 12      | 80         | 87.0          | 81.5         | 5.5          | 26           |              |                | 30                                  |
| 13      | 76         | 91.0          | 84.0         | 7.0          | 24           |              |                | 20                                  |
| 14      | 81         | 98.0          | 95.5         | 2.5          | 6            | 98.0         | 8              | 25                                  |
| 15      | 89         | 97.0          | 93.0         | 4.0          | 20           | 97.0         | 10             | 25                                  |
| 17      | 76         | 96.0          | 89.0         | 7.0          | 25           | 95.0         | 22             | 30                                  |
| 18      | 87         | 98.0          | 98.0         | 0.0          | 20           |              |                | 20                                  |
| 19      | 80         | 89.0          | 87.0         | 2.0          | 10           | 89.0         | 12             | 15                                  |
| 22      | 76         | 88.0          | 83.0         | 5.0          | 22           |              |                | 15                                  |
| 23      | 76         | 86.0          | 75.0         | 11.0         | 50           |              |                | 15                                  |
| 24      | 86         | 98.0          | 96.0         | 2.0          | 10           | 98.0         | 25             | 20                                  |
| 26      | 80         | 95.0          | 93.0         | 2.0          | 6            | 96.0         | 7              | 25                                  |
| 27      | 76         | 98.0          | 96.0         | 2.0          | 5            | 98.0         | 10             | 10                                  |
| 31      | 80         | 98.0          | 94.0         | 0.0          | 10           | 98.0         | 20             | 15                                  |
| 32      | 72         | 80.0          | 88.0         | +8.0         | 10           |              |                | 10                                  |
| 33      | 72         | 92.0          | 91.0         | 2.0          | 18           |              |                | 15                                  |
| 34      | 82         | 86.0          | 86.0         | 0.0          | 15           |              |                | 5                                   |

TABLE II  
HABITUAL SMOKERS TESTED AT DIFFERENT TIMES

| SUBJECT | ROOM TEMP. | INITIAL TEMP. | LOWEST TEMP. | DEGREES DROP | TIME OF DROP | RETURN TEMP. | TIME OF RETURN |
|---------|------------|---------------|--------------|--------------|--------------|--------------|----------------|
|         |            |               |              |              | min.         |              | min.           |
| 1       | 63         | 75            | 72.5         | 2.5          | 12           | 91           | 14             |
|         | 67         | 85            | 80.6         | 4.4          | 8            | 86           | 5              |
| 2       | 72         | 79            | 76.0         | 3.0          | 10           | 83           | 20             |
|         | 72         | 81            | 78.0         | 3.0          | 12           | 81           | 6              |
| 5       | 70         | 87            | 76.0         | 11.0         | 20           | 78           | 5              |
|         | 72         | 93            | 88.0         | 5.0          | 8            | 92           | 10             |
| 7       | 68         | 86            | 79.5         | 6.5          | 12           | 87           | 8              |
|         | 68         | 87            | 81.5         | 5.5          | 17           | 89           | 14             |

TABLE III  
NONSMOKERS

| SUBJECT | ROOM TEMP. | INITIAL TEMP. | LOWEST TEMP. | DEGREES DROP | TIME OF DROP | RETURN TEMP. | TIME OF RETURN |
|---------|------------|---------------|--------------|--------------|--------------|--------------|----------------|
|         |            |               |              |              |              | min.         | min.           |
| 9       | 66         | 88            | 86.0         | 2.0          | 6            | 91           | 5              |
| 16      | 79         | 96            | 97.0         | +1.0         | 20           |              |                |
| 20      | 83         | 90            | 86.5         | 3.5          | 20           | 92           | 18             |
| 21      | 86         | 98            | 98.0         | 0.0          | 14           |              |                |
| 25      | 81         | 97            | 97.0         | 0.0          | 50           |              |                |
| 28      | 97         | 100           | 100.0        | 0.0          | 20           |              |                |
| 29      | 88         | 98            | 94.0         | 4.0          | 8            | 97           | 12             |
| 30      |            | 98            | 98.0         | 0.0          | 10           |              |                |



In 4 cases the minimum temperature was reached and the return elevation of temperature began while the subject was still smoking.

There was apparently no relation between the number of cigarettes consumed daily by the subject and his temperature reaction.

TABLE IV  
THROMBOANGIITIS OBLITERANS

| SUBJECT | ROOM TEMP. | INITIAL TEMP. | LOWEST TEMP. | DEGREES DROP | TIME OF DROP<br>min. | RETURN TEMP. | TIME OF RETURN<br>min. |
|---------|------------|---------------|--------------|--------------|----------------------|--------------|------------------------|
| 13      | 76         | 91            | 84           | 7            | 24                   |              |                        |
| 17      | 76         | 96            | 89           | 7            | 25                   | 95           | 22                     |
| 23      | 82         | 86            | 75           | 11           | 50                   |              |                        |
| 36      | 80         | 86            | 86           | 0            | 15                   |              |                        |

In comparing the group of smokers with the nonsmokers, it will be noted that 88 per cent of the smokers showed an average drop in skin temperature of 4.3° F. In the nonsmoker group only 36 per cent showed a drop and this average was 3.2° F. Conditions were identical for both groups. It was noted, however, that the nonsmokers did not inhale. Earp<sup>7</sup> has pointed out that inhalers take 8 times as much nicotine into their systems as smokers who do not inhale. Winterstein and Aronson showed that from 2.5 per cent to 4.4 per cent of the nicotine is absorbed on smoking without inhaling and from 8.1 per cent to 17 per cent is absorbed when inhaled. When we consider that in the act of inhaling, the smoke is exposed to the mucous membranes of the nasopharynx and the lower respiratory passages, thus giving an infinitely larger surface on which the products of smoking may condense, we may expect a greater deposition and consequent absorption of nicotine and other components, than would result from simply puffing the smoke in and out of the mouth. This may be the explanation for the marked difference between the temperature changes of the smokers as compared with the nonsmokers.

In personal communication with Dr. Wright, pertaining to his work on capillary activity during active smoking, he states:

"In certain individuals we have been able to note a marked slowing and often stasis in the capillary circulation at the nailfold during the smoking of a single cigaret. This phenomenon when present can be seen after the first few deep inhalations. It has been found especially in subjects who complain of systemic symptoms from the use of tobacco."

#### COMMENT

As has been repeatedly contended during the past few decades, this work further tends to confirm the opinion that smoking does bring about a peripheral vasoconstriction, which may be a significant contributing etiologic factor in certain of the vascular disorders and particularly in thromboangiitis obliterans.

NOTE: Since this manuscript was prepared, Haggard and Greenberg<sup>8</sup> have shown that an increased blood sugar results from smoking, and they attribute this to adrenal stimulation by nicotine through the sympathetics. This has been confirmed by the present writers<sup>9</sup> who also believe that the vasoconstriction described above can probably be attributed to the same cause.

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VITAMIN THERAPY IN PULMONARY TUBERCULOSIS\*

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VI. THE EFFECT OF VIOSTEROL ON THE CARBON DIOXIDE CONTENT, THE H-10% CONCENTRATION, CHLORIDES, GLUCOSE AND UREA NITROGEN OF THE BLOOD, AND PROTEIN, CALCIUM, AND PHOSPHORUS OF THE SERUM. THE EFFECT OF PHYSIOLOGIC SALINE ON THESE CONSTITUENTS DURING THE STATE OF HYPERCALCEMIA.

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IN PREVIOUS experiments<sup>1</sup> we demonstrated the effect of viosterol on the calcium and phosphorus metabolism, and the effect of intravenous physiologic saline upon these two elements. In order to determine the effect of viosterol upon other constituents of the blood we performed simultaneously the following chemical analyses before, during, and after hypercalcemia. Likewise, the same procedures were employed in determining the effect of physiologic saline upon these constituents when administered during hypercalcemia. These analyses were performed on 15 patients who had a diagnosis of pulmonary tuberculosis, Stages II and III.

## METHODS

Serum calcium was determined by the Clark and Collip<sup>2</sup> method, modification of Kramer and Tisdall.<sup>3</sup> Phosphorus determinations were made by the method of Benedict and Theis.<sup>4</sup> McDonald's<sup>5</sup> modification of Hastings and Sendroy's<sup>6</sup> method was used in making the analyses of the P<sub>H</sub>. Carbon dioxide was determined by the volumetric method of Van Slyke;<sup>7</sup> urea nitrogen according to the method of Myers;<sup>8</sup> glucose by the micromethod of Folin;<sup>9</sup> chlorides by the method of Whitehorn;<sup>10</sup> and albumin and globulin by the method of

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\*From Boehne Tuberculosis Hospital.

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Wu and Ling<sup>11</sup> and Greenberg,<sup>12</sup> respectively. If the albumin determination is allowed to incubate four or five hours, very little difference in the result is obtained, whereas the globulin may vary considerably after two hours.

#### THE EFFECT OF VIOSTEROL ON SERUM CALCIUM AND PHOSPHORUS

Data presented here further corroborate the earlier work<sup>13, 14</sup> with viosterol 250D\* and 10,000X.† The solution used in this experiment had a cod liver oil coefficient of 10,000, which is one hundred times stronger than 250D. The daily dose was 0.5 c.c. (20 gts.) which is the rat assay equivalent in vitamin D of 5,000 c.c. of cod liver oil. The average period of viosterol administration for this group was approximately eight days. A condition of hypercalcemia as a result of this dosage was brought about between the eighth and fifteenth days. Serum calcium concentrations averaged from 13.3 to 18.4 mg. per 100 c.c. The average normal serum calcium concentration was 11.4 mg. per 100 c.c. for the 15 cases (Table I). The average serum calcium after the period of viosterol administration was 15.2 mg., an average elevation of 3.8 mg. per 100 c.c. The average serum phosphorus concentration was 4.7 mg. per 100 c.c. before, and 4.9 mg. per 100 c.c. after the viosterol administration. It is significant to note that this concentration of phosphorus in the blood serum would have been elevated had it been determined during the first five days of viosterol administration.<sup>14</sup> However, if one determines the serum phosphorus after the serum calcium has reached a higher value, the serum phosphorus is reciprocally lower.

We have demonstrated<sup>1</sup> that physiologic saline lowers the concentration of calcium and phosphorus in the blood, and this work further verifies this specificity. Within twelve hours after the administration of between 3,000 and 5,000 c.c. of physiologic saline per individual, the serum calcium concentration dropped to 12.7 mg. per 100 c.c. (Table I), an average decrease of 2.5 mg. Serum phosphorus was decreased by physiologic saline as shown by the average of 3.7 mg. per 100 c.c., a decline of 1.2 mg.

The lowering of serum calcium and phosphorus is not due to a change in blood volume. Since determinations were made twelve hours after the administration of saline, blood volume would have returned to normal. Smith and Mendel<sup>15</sup> injected into the veins of rabbits isotonic solutions of various neutral sodium salts. The blood volume was doubled although they found that different salts were eliminated from the blood at different rates. Most of the fluid in every case passed into the tissues within five minutes without causing evident edema. If the decrease in serum calcium and phosphorus was due to dilution of the blood volume, a return to the original high concentrations would be expected after the effect of saline had passed.

#### THE EFFECT OF VIOSTEROL ON $P_H$ AND $CO_2$

The average  $P_H$  for the 15 cases was 7.39 prior to the administration of viosterol. This average is 0.02 more than the  $P_H$  of 50 normal individuals who equaled 7.37.<sup>16</sup> After viosterol the average  $P_H$  of the blood for the 15 cases was 7.41. The effect of a viosterol hypercalcemia upon the hydrogen ion concen-

\*10,000 International units per gram.

†10,000 times average cod liver oil, 1,000,000 International units per gram; kindly supplied by Mead Johnson and Company.

TABLE I

| CASE  | SERUM            |             |                  |                  |                  |             | BLOOD                                    |                  |        |                  |        |                  |                      |             |        |                  |        |             |                  |                  |        |             |     |     |    |    |    |    |
|-------|------------------|-------------|------------------|------------------|------------------|-------------|------------------------------------------|------------------|--------|------------------|--------|------------------|----------------------|-------------|--------|------------------|--------|-------------|------------------|------------------|--------|-------------|-----|-----|----|----|----|----|
|       | CALCIUM          |             | PHOSPHORUS       |                  | PROTEIN          |             | CARBON DIOX-<br>IDE COMBIN-<br>ING POWER |                  |        | P <sub>H</sub>   |        |                  | CHLORIDES AS<br>NaCl |             |        | GLUCOSE          |        |             | UREA<br>NITROGEN |                  |        |             |     |     |    |    |    |    |
|       | MG. PER 100 C.C. |             | MG. PER 100 C.C. |                  | GM. PER 100 C.C. |             | ALBUMIN                                  |                  |        | MG. PER 100 C.C. |        |                  | MG. PER 100 C.C.     |             |        | MG. PER 100 C.C. |        |             | MG. PER 100 C.C. |                  |        |             |     |     |    |    |    |    |
|       | NORMAL           | AFTER NaCl† | NORMAL           | AFTER VIOSTEROL* | NORMAL           | AFTER NaCl† | NORMAL                                   | AFTER VIOSTEROL* | NORMAL | AFTER NaCl†      | NORMAL | AFTER VIOSTEROL* | NORMAL               | AFTER NaCl† | NORMAL | AFTER VIOSTEROL* | NORMAL | AFTER NaCl† | NORMAL           | AFTER VIOSTEROL* | NORMAL | AFTER NaCl† |     |     |    |    |    |    |
| 1134  | 11.7             | 18.4        | 13.5             | 4.8              | 5.2              | 3.6         | 3.10                                     | 2.76             | 2.10   | 4.20             | 4.48   | 4.15             | 67                   | 61          | 69     | 7.37             | 7.42   | 7.37        | 468              | 410              | 546    | 100         | 88  | 100 | 75 | 15 | 22 | 12 |
| 1129  | 11.5             | 14.7        | 12.7             | 4.4              | 4.4              | 3.4         | 2.80                                     | 2.70             | 1.92   | 4.20             | 4.70   | 3.99             | 62                   | 64          | 65     | 7.42             | 7.46   | 7.47        | 476              | 460              | 530    | 88          | 100 | 113 | 17 | 20 | 18 | 18 |
| 1130  | 10.8             | 16.6        | 14.5             | 4.4              | 5.6              | 2.9         | 3.54                                     | 3.29             | 2.50   | 4.00             | 4.20   | 3.47             | 60                   | 60          | 66     | 7.40             | 7.42   | 7.40        | 410              | 388              | 452    | 88          | 88  | 88  | 13 | 18 | 11 | 11 |
| 1115  | 12.6             | 15.0        | 12.9             | 4.4              | 4.3              | 3.7         | 2.17                                     | 2.70             | 2.26   | 3.77             | 4.52   | 3.31             | 61                   | 62          | 53     | 7.40             | 7.42   | 7.42        | 482              | 480              | 500    | 113         | 88  | 100 | 12 | 9  | 13 | 13 |
| 686   | 12.0             | 13.9        | 12.8             | 5.0              | 4.3              | 2.8         | 2.80                                     | 2.50             | 2.71   | 3.59             | 4.10   | 3.99             | 57                   | 68          | 52     | 7.37             | 7.37   | 7.40        | 480              | 434              | 476    | 113         | 88  | 113 | 14 | 17 | 16 | 16 |
| 1012  | 12.1             | 17.1        | 13.6             | 4.7              | 5.2              | 2.8         | 2.78                                     | 2.59             | 1.77   | 3.67             | 4.27   | 3.24             | 57                   | 58          | 64     | 7.42             | 7.40   | 7.42        | 452              | 428              | 488    | 88          | 75  | 63  | 12 | 18 | 10 | 10 |
| 1109  | 12.4             | 15.4        | 13.2             | 5.3              | 5.6              | 3.6         | 2.80                                     | 2.30             | 2.50   | 3.80             | 4.80   | 4.14             | 58                   | 60          | 43     | 7.40             | 7.40   | 7.52        | 450              | 420              | 516    | 113         | 100 | 113 | 11 | 15 | 12 | 12 |
| 1068  | 12.4             | 13.6        | 12.2             | 5.7              | 6.2              | 4.6         | —                                        | —                | —      | —                | —      | —                | 57                   | 66          | 52     | 7.42             | 7.40   | 7.42        | 484              | 420              | 525    | 125         | 100 | 110 | 12 | 15 | 12 | 12 |
| 614   | 10.9             | 13.3        | 11.9             | 4.5              | 4.3              | 3.1         | —                                        | —                | —      | —                | —      | —                | 46                   | 61          | 57     | 7.42             | 7.40   | 7.42        | 468              | 460              | 500    | 125         | 200 | 113 | 10 | 12 | 9  | 9  |
| 1047  | 10.3             | 16.0        | 12.3             | 5.3              | 4.6              | 4.6         | —                                        | —                | —      | —                | —      | —                | 53                   | 63          | 58     | 7.36             | 7.37   | 7.42        | 450              | 404              | 510    | 145         | 125 | 125 | 11 | 15 | 11 | 11 |
| 966   | 11.8             | 14.0        | 12.6             | 3.4              | 4.2              | 3.3         | —                                        | —                | —      | —                | —      | —                | 52                   | 64          | 50     | 7.38             | 7.40   | 7.42        | 500              | 452              | 478    | 112         | 138 | 112 | 10 | 13 | 10 | 10 |
| 958   | 11.1             | 15.7        | 13.5             | 3.7              | 4.0              | 3.0         | —                                        | —                | —      | —                | —      | —                | 58                   | 61          | 54     | 7.39             | 7.41   | 7.42        | 500              | 439              | 488    | 114         | 100 | 88  | 12 | 13 | 14 | 14 |
| 1156  | 10.9             | 13.3        | 11.5             | 4.9              | 4.6              | 4.1         | 2.80                                     | 1.87             | 2.09   | 4.89             | 6.18   | 3.98             | 56                   | 52          | 59     | 7.42             | 7.47   | 7.45        | 429              | 412              | 445    | 75          | 88  | 175 | 12 | 17 | 9  | 9  |
| 1146  | 10.6             | 16.3        | 12.5             | 5.5              | 6.6              | 5.0         | 2.88                                     | 2.59             | 2.80   | 3.71             | 4.22   | 3.86             | 57                   | 48          | 54     | 7.37             | 7.45   | 7.35        | 464              | 445              | 545    | 75          | 113 | 113 | 18 | 17 | 14 | 14 |
| 1140  | 9.4              | 15.4        | 11.5             | 4.4              | 5.6              | 5.3         | 2.88                                     | 2.48             | 2.20   | 4.68             | 4.14   | 4.08             | 63                   | 62          | 65     | 7.37             | 7.37   | 7.40        | 368              | 368              | 400    | 63          | 88  | 75  | 22 | 22 | 18 | 18 |
| Total | 11.4             | 15.2        | 12.7             | 4.7              | 4.9              | 3.7         | 2.85                                     | 2.58             | 2.28   | 4.05             | 4.56   | 3.81             | 59                   | 61          | 57     | 7.39             | 7.41   | 7.42        | 459              | 428              | 493    | 102         | 105 | 105 | 14 | 16 | 13 | 13 |
| Av.   | 11.4             | 15.2        | 12.7             | 4.7              | 4.9              | 3.7         | 2.85                                     | 2.58             | 2.28   | 4.05             | 4.56   | 3.81             | 59                   | 61          | 57     | 7.39             | 7.41   | 7.42        | 459              | 428              | 493    | 102         | 105 | 105 | 14 | 16 | 13 | 13 |

\*Viosterol 10,000 X, 0.5 c.c. (20 ctt.) administered between 12:00 and 1:00 p.m.

\*Viosterol 10,000 X, 0.5 c.c. (20 gtt.) administered between eight and fifteen days. Determination made on day drug was discontinued.  
†After NaCl, determinations made twelve hours later.

tration of the blood was shown as a slight shift toward the alkaline side. Individual variations of fever may have accounted for an increased  $P_H$  in an occasional case. This is especially true after the administration of physiologic saline. This is demonstrated in Case 1,109, Table I. The average  $P_H$  for the same group after the intravenous injection of physiologic saline was 7.42. The large quantity of saline used in a short period of time had no deleterious effect on the patients outside of an occasional chill.

These data show that viosterol hypercalcemia is not accompanied by an increase in hydrogen ion concentration. In hypercalcemia produced by viosterol our data do not conform to the opinion quoted by Peters and Van Slyke,<sup>17</sup> that a lower  $P_H$  tends to mobilize the calcium in the blood stream. Bernheim<sup>18</sup> states that diffusible calcium increased with a decrease in  $P_H$ . The average for 10<sup>19</sup> of the 15 cases (5 additional ones in this report) presented in Table I shows an increase in both diffusible and nondiffusible calcium during the state of hypercalcemia when the  $P_H$  was increased. This work demonstrated that viosterol increased the nondiffusible calcium to a much greater extent than the diffusible calcium. At the same time the hydrogen ion concentration was decreased ( $P_H$  increased).

Carbon dioxide in this series of 15 cases averaged 59 volumes per cent. During the period of hypercalcemia the average carbon dioxide percentage was 61. Although this percentage is within normal limits it points to a slight increase in carbon dioxide during the state of hypercalcemia. Following the administration of physiologic saline, carbon dioxide averaged 57 volumes per cent. A decrease from 61 to 57 volumes per cent can be accounted for by fever. Collip and Backus<sup>20</sup> were the first to point out that overventilation produces an alkalosis due to washing out of carbon dioxide from the blood. This fact was later borne out by Grant and Goldman.<sup>21</sup> It was further noted by Koehler<sup>22</sup> that acute clinical fevers produce a similar picture, namely, a low  $CO_2$  and a high  $P_H$ .

#### THE EFFECT OF VIOSTEROL ON GLOBULIN AND ALBUMIN

In 10 cases (Table I) the globulin and albumin were analyzed simultaneously with the other determinations. The total protein average was found to be 6.90 gm. per 100 c.c.; globulin was 2.85 gm.; albumin was 4.05 gm. During hypercalcemia the total protein increased to 7.14 gm. per 100 c.c. This increase was due to the elevation of albumin. This is in accord with the finding of other investigators,<sup>23</sup> namely, that proteins permit serum to hold more calcium in solution. When calcium was increased by means of viosterol, it was the albumin fraction which was responsible for retention of the calcium. Globulin decreased during viosterol hypercalcemia. Following the administration of physiologic saline intravenously, the total protein decreased to 6.10 gm. per 100 c.c. Globulin and albumin decreased, the albumin more than the globulin. Further work is required to explain the mechanism by which protein is diminished following administration of intravenous physiologic saline.

#### THE EFFECT OF VIOSTEROL ON CHLORIDES, GLUCOSE AND UREA NITROGEN

The 15 cases (Table I) showed a normal average of 459 mg. of chlorides preceding the administration of viosterol. During hypercalcemia, the chlorides

averaged 428 mg. per 100 c.c. All but two cases showed a decrease in chlorides. During the intravenous administration of physiologic saline the chlorides were elevated. The average for the 15 cases was 493 mg. per 100 c.c., an increase of 65 mg. per 100 c.c. over the average during the condition of hypercalcemia, and an increase of 44 mg. per 100 c.c. over the normal average.

The average glucose concentration of the blood for the 15 cases was 102 mg. per 100 c.c. After the administration of viosterol the glucose was found to average 105 mg. per 100 c.c. Following the administration of physiologic saline, the average glucose was 105 mg. per 100 c.c. These data present a slight increase in glucose after viosterol. Physiologic saline had no effect on the glucose of the blood.

Although some cases of pulmonary tuberculosis tend to have a slightly elevated urea nitrogen, the average for this group of cases was 14 mg. per 100 c.c. During hypercalcemia, the urea nitrogen showed a tendency to increase in this series of cases. Following the administration of physiologic saline, the urea nitrogen averaged 13 mg. per 100 c.c. Sweany, Weathers and McCluskey<sup>21</sup> observed that in tuberculosis with no serious kidney lesion present, the chlorides and urea nitrogen played a reciprocal rôle. In this series when the chlorides were increased by physiologic saline, the urea nitrogen was slightly decreased.

#### SUMMARY

1. Viosterol increases the total protein, calcium, and phosphorus in the blood. The albumin fraction of the protein is increased, globulin decreased. The administration of intravenous physiologic saline decreased the total protein, calcium, and phosphorus.

2. The state of hypercalcemia produced by viosterol is not accompanied by an increased hydrogen ion concentration ( $P_H$  decreased).

3. The chloride content of the blood is slightly decreased during viosterol hypercalcemia, and the urea nitrogen is very slightly increased.

4. The carbon dioxide content and glucose are not significantly altered by the massive doses of viosterol used to produce hypercalcemia.

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## CHEMOTHERAPEUTIC STUDIES WITH SODIUM RICINOLEATE (SORICIN)\*

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**I**N CHEMOTHERAPY and especially of bacterial infections the possibility of producing chemical agents capable of inactivating or destroying toxins and related substances in the blood and other tissues has long been of engaging interest. Of course, the predominating motive of chemotherapeutic research is the production of chemical agents possessing parasiticial activity *in vivo*, but almost of equal importance is the possibility of producing compounds of detoxifying activity *in vivo* even though lacking in demonstrable parasiticial activity.

This is especially true in many of the bacterial infections not only because exogenous toxins are the chief pathogenic agents in some like diphtheria, tetanus, gangrene, scarlet fever, and erysipelas, but because bacterial products, both toxic and nontoxic, produced in the tissues during infection, would appear capable of retarding or preventing phagocytosis and preventing the activity of humoral antibodies. In the case of some bacterial infections these agents like the aggressins do not appear to be primarily toxic but are, nevertheless, pathogenic in this sense of retarding or preventing phagocytosis. In pneumococcus pneumonia for example, it would appear that the nontoxic and nonantigenic soluble substance "S" discovered by Avery and his coworkers has the faculty of neutralizing the antibodies produced by the patient and enhancing the virulence of the organisms by preventing their phagocytosis.

At the present time our main hope of combating the toxins and these nontoxic agents is by means of specific immune sera but the possibility of discovering chemical agents capable of inactivating or completely destroying them *in vivo* is of fundamental importance.

From this standpoint we have been greatly interested in sodium ricinoleate, that is, in the possibility of this compound destroying these toxic and nontoxic bacterial agents *in vivo* and especially since the investigations of Larson and his colleagues,<sup>1</sup> Sommer and Sommer,<sup>2</sup> Netter, et al.,<sup>3</sup> Dorst and Morris<sup>4</sup> and others, the literature being briefly summarized by Harris and Bunker,<sup>5</sup> have indicated that sodium ricinoleate is capable of neutralizing various bacterial toxins, phytotoxins and cobra venom in the test tube and possibly in the living animal in the case of some of them with special reference to streptococci and pneumococci.

Furthermore in chemotherapeutic research there is always the possibility of compounds inert in the test tube undergoing transformation in the living

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animal into new and more active antiparasiticideal agents. For this reason test tube bactericideal activity *in vitro* may be without curative activity in the living animal or, being extremely low in parasiticideal activity in the test tube, undergo transformation in the blood or tissues into compounds of extremely high curative activity as in the case of arsphenamine, neoarsphenamine, and other trivalent organic arsenical compounds. For these reasons test tube determinations of bactericideal activity are of most value in the case of those compounds to be used in treatment by local application, whereas in the case of those designed for parenteral administration actual tests with experimental infections in living animals are required. For example, we think it is extremely fortunate that Ehrlich and his colleagues did not have cultures of trypanosomes and *Spirocheta pallida* available in 1907, for they may have committed the error of testing their new arsenical compounds, including salvarsan, in the test tube only and finding them so low in parasiticideal activity may have neglected their administration to infected animals and thereby missed or delayed making their great discoveries. Therefore, even though we knew sodium ricinoleate to be of very low bactericideal activity in the test tube, we have thought it worth while to administer it in the treatment of various experimental infections and especially since other investigators have reported results lending some encouragement to these efforts.

Furthermore the studies of Larson and his colleagues and others have indicated the possibility of sodium ricinoleate transforming some of the bacterial toxins into toxoids or other compounds of greatly reduced toxicity in the test tube but retaining their antigenic properties, and thereby of interest from the standpoint of vaccination against diphtheria, scarlet fever, erysipelas, etc. But this is a study in immunity rather than in chemotherapy and has not engaged our particular interest in these investigations, the results of which are briefly summarized in this communication.

#### TOXICITY OF SODIUM RICINOLEATE

The compound employed in this study<sup>2</sup> was prepared by Rider<sup>6</sup> with a high degree of purity and its toxicity determined by intravenous, intraperitoneal, intramuscular and subtheal administration preliminary to trial in the treatment of several experimental bacterial septicemias and toxinemias, trypanosomiasis, syphilis, and poliomyelitis of the lower animals.

(a) *By Intravenous Injection*.—When 2 per cent solutions were given rats intravenously with a gravity apparatus at the rate of 1 c.c. in two minutes, as employed in determining the toxicity of arsphenamine and neoarsphenamine,<sup>7</sup> the maximum tolerated dose permitting animals to survive ten days or longer was approximately 0.035 gm. per kilogram weight (Table I) while 0.050 gm. per kilogram produced death immediately or within one or two days (minimal lethal dose). It is, therefore, about 4 times as toxic as alkalized solutions of arsphenamine by this route of administration. When the injections were given more rapidly and especially with more concentrated solutions, the tolerance was greatly reduced, showing that for this route of administration it is necessary to use more dilute solutions slowly injected.

<sup>2</sup>Kindly furnished by the William S. Merrell Company of Cincinnati, Ohio.

Rabbits have borne 10 daily doses of 0.010 gm. per kilogram and 0.005 gm. per kilogram in as many as 30 consecutive daily doses without untoward effects, except the production of sclerosis and thrombosis of the marginal ear veins and microscopic examination of the lungs, heart, liver, and kidneys have shown practically no histologic evidences of injury. However, the heart and lungs of rabbits and rats succumbing immediately after the injection of lethal doses have shown large emboli of hemolyzed erythrocytes, the whole histologic picture bearing a striking resemblance to those changes described by Kolmer and Lucke<sup>8</sup> following the intravenous injection of acid solutions of arsphenamine, producing intravascular hemolysis and agglutination.

TABLE I  
TOXICITY OF SODIUM RICINOLEATE

| ANIMAL     | ROUTE OF ADMINISTRATION | SOLUTION | MINIMAL LETHAL DOSE PER KILO | MAXIMUM TOLERATED DOSE PER KILO |
|------------|-------------------------|----------|------------------------------|---------------------------------|
| Rat        | Intraven.               | 2.0%     | 0.050 gm.                    | 0.035 gm.*                      |
| Rat        | Intramus.               | 10.0%    | —                            | More than 1.0 gm.               |
| Rat        | Intraperit.             | 4.0%     | 0.5 gm.                      | 0.3 gm.                         |
| Mice       | Intraperit.             | 4.0%     | 0.5 gm.                      | 0.25 gm.                        |
| Guinea pig | Intraperit.             | 4.0%     | —                            | 0.16 gm.                        |
| Guinea pig | Intraspinal             | 0.1%     | —                            | 0.004 gm.                       |

\*Rabbits have tolerated 10 daily doses of 0.010 gm. and 30 daily doses of 0.005 gm. per kilogram without untoward effects.

TABLE II  
HEMOLYTIC ACTIVITY OF SODIUM RICINOLEATE IN WATER-BATH AT 37° C.

| WASHED ERYTHROCYTES |               |            |              | WHOLE DEFIBRINATED BLOOD |        |            |              |
|---------------------|---------------|------------|--------------|--------------------------|--------|------------|--------------|
| SODIUM RICINOLEATE  | 5% CORPUSCLES | FINAL DIL. | HEMOLYSIS    | SODIUM RICINOLEATE       | BLOOD  | FINAL DIL. | HEMOLYSIS    |
| 1 c.c. 1: 25        | 1 c.c.        | 1: 50      | At once      | 1 c.c. 1: 25             | 1 c.c. | 1: 50      | At once      |
| 1 c.c. 1: 50        | 1 c.c.        | 1: 100     | At once      | 1 c.c. 1: 50             | 1 c.c. | 1: 100     | At once      |
| 1 c.c. 1: 100       | 1 c.c.        | 1: 200     | Few seconds  | 1 c.c. 1: 100            | 1 c.c. | 1: 200     | Few seconds  |
| 1 c.c. 1: 200       | 1 c.c.        | 1: 400     | Few seconds  | 1 c.c. 1: 200            | 1 c.c. | 1: 400     | Few seconds  |
| 1 c.c. 1: 400       | 1 c.c.        | 1: 800     | 15 seconds   | 1 c.c. 1: 400            | 1 c.c. | 1: 800     | About minute |
| 1 c.c. 1: 800       | 1 c.c.        | 1:1,600    | About minute | 1 c.c. 1: 800            | 1 c.c. | 1:1,600    | 20 minutes   |
| 1 c.c. 1:1,600      | 1 c.c.        | 1:3,200    | About minute | 1 c.c. 1:1,600           | 1 c.c. | 1:3,200    | Incomplete   |
| 1 c.c. 1:3,200      | 1 c.c.        | 1:6,400    | 30 minutes   | 1 c.c. 1:3,200           | 1 c.c. | 1:6,400    | Incomplete   |

(b) *Hemolytic and Agglutinating Activity.*—Indeed 1 c.c. of 1:3200 solution of sodium ricinoleate in saline solution is capable of completely hemolyzing 1 c.c. of 5 per cent suspension of washed rabbit corpuscles (final dilution 1:6400) in about thirty minutes at 37° C. (Table II). Even in the presence of plasma, the hemolytic activity of the compound is quite high, since 1 c.c. of 1:800 dilution has produced complete hemolysis of 1 c.c. of defibrinated rabbit blood (final dilution 1:1600) within twenty minutes in a water-bath at 37° C.

Curiously, however, sodium ricinoleate does not produce any marked degree of agglutination of either washed or unwashed erythrocytes in the test tube, and it would appear that intravascular agglutination plays little or no part in toxicity by intravenous injection, although it may be that the emboli found in the heart and vessels of the lungs as well as to some extent in the kidneys are

due to thromboplastic stromas and other debris of hemolysis as suggested by Gerwe<sup>2</sup> and responsible for the respiratory and cardiac failure resulting in death following intravenous injections of lethal amounts.

(c) *By Intramuscular Injection.*—By intramuscular injection, however, the hemolytic and indirect thromboplastic effects of sodium ricinoleate are largely removed and consequently the toxicity is greatly reduced, since rats have withstood doses larger than 1.0 gm. per kilogram of weight (Table I). Not infrequently, however, abscesses have resulted at the sites of injection showing that the compound produces considerable inflammation with liquefaction necrosis by this route of administration, the pus being frequently of a brownish color due to local hemolysis.

(d) *By Intraperitoneal Injection.*—The same has been found true after intraperitoneal injections in rats, mice, and guinea pigs in that upon autopsy extensive inflammatory changes of the parietal and visceral peritoneum was sometimes observed along with light reddish-brown exudates of a soapy nature. Indeed rats and mice usually succumbed within twenty-four hours to doses of 0.5 gm. per kilogram (minimal lethal dose) although single doses as high as 0.25 to 0.3 gm. per kilogram (Table I) were borne indefinitely (maximum tolerated dose). With guinea pigs, however, the maximum tolerated dose was somewhat less being about 0.16 gm. per kilogram which corresponds quite closely to the dose of 0.12 to 0.27 gm. per kilo observed by Gerwe.<sup>2</sup>

(e) *By Intracisternal Injection.*—Since we proposed using sodium ricinoleate in the treatment of tetanus of guinea pigs by intraspinal injection it was necessary to determine its toxicity by this route of administration, and in guinea pigs it was found that the maximum tolerated dose by intracisternal injection was approximately 0.004 gm. per kilo or about one-tenth of the maximum tolerated dose by intravenous injection (Table I).

#### THE EFFECT OF SODIUM RICINOLEATE UPON DIPHTHERIA TOXIN

Along with a large number of other compounds<sup>5</sup> sodium ricinoleate is capable of inactivating diphtheria toxin in the test tube. As shown in Table III as an example of the results of several experiments, an amount as small as 0.0075 gm. dissolved in 1 c.c. of saline solution and added to 6 M. L. D. of diphtheria toxin in 1 c.c. is capable of completely inactivating the latter during an exposure of one hour at room temperature as determined by the subcutaneous injection of the 2 c.c. mixtures into young guinea pigs (8 to 10 ounces). When, however, an L+ dose of toxin is employed, amounts of sodium ricinoleate ranging from 0.003 to 0.030 gm. are unable to completely detoxify this large amount of toxin in the test tube.

Whether or not the detoxified mixtures are capable of engendering immunity, that is, of retaining antigenic activity, has not been definitely determined. In one experiment we partially detoxified 5 L+ doses with 0.1 gm. of sodium ricinoleate dissolved in 50 c.c. of saline and allowed to stand in an incubator for one hour and kept in a refrigerator. Twelve guinea pigs were subsequently given 1 c.c. subcutaneously every five days. Of these 8 died after 5 to 8 injections due to incomplete detoxification of the toxin. Four survived

and three weeks after the last dose were given a subcutaneous injection of 3 M. L. D.'s of toxin sufficient for producing fatal intoxication of 4 controls on the third day after injection; the immunized pigs succumbed in from six to eight days, so that it would appear that but little immunity had been engendered and suggesting that sodium ricinoleate may de-antigenize a portion of diphtheria toxin in vitro.

TABLE III  
THE EFFECT OF SODIUM RICINOLEATE UPON DIPHTHERIA TOXIN IN VITRO

| DIPHTHERIA TOXIN<br>(1 C.C.) | SODIUM<br>RICINO-<br>LEATE<br>(GM.) | RESULTS IN DAYS |   |   |   |   |   |   |   |   |    |    |
|------------------------------|-------------------------------------|-----------------|---|---|---|---|---|---|---|---|----|----|
|                              |                                     | 1               | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 12 |
| 1 L + dose                   | 0.003                               | —*              | D |   |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.003                               | —               | D |   |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.006                               | —               | — | D |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.006                               | —               | — | — | D |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.0075                              | —               | D |   |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.0075                              | —               | — | — | D |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.010                               | —               | D |   |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.010                               | —               | D |   |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.015                               | —               | — | D |   |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.015                               | —               | — | — | D |   |   |   |   |   |    |    |
| 1 L + dose                   | 0.030                               | —               | — | D |   |   |   |   |   |   |    |    |
| 6 M. L. D.                   | 0.030                               | —               | — | — | — | D |   |   |   |   |    |    |
| 6 M. L. D.                   | 0.003                               | —               | — | — | — | D |   |   |   |   |    |    |
| 6 M. L. D.                   | 0.006                               | —               | — | — | — | — |   |   |   |   |    |    |
| 6 M. L. D.                   | 0.0075                              | —               | — | — | — | — | — |   | D |   |    |    |
| 6 M. L. D.                   | 0.010                               | —               | — | — | — | — | — | — | — | — | —  | —  |
| 6 M. L. D.                   | 0.015                               | —               | — | — | — | — | — | — | — | — | —  | —  |
| 6 M. L. D.                   | 0.030                               | —               | — | — | — | — | — | — | — | — | —  | —  |
| Control (1 L +)              | 0                                   | —               | D |   |   |   |   |   |   |   |    |    |
| Control (1 L +)              | 0                                   | —               | D |   |   |   |   |   |   |   |    |    |
| Control (6 M.L.D.)           | 0                                   | —               | D |   |   |   |   |   |   |   |    |    |
| Control (6 M.L.D.)           | 0                                   | —               | D |   |   |   |   |   |   |   |    |    |

\* — = survived: D = died.

However, of much more interest from the standpoint of chemotherapy is the question of whether or not sodium ricinoleate is capable of destroying diphtheria toxin in the living guinea pig.

For this purpose animals were given subcutaneous injections of 6 M. L. D.'s of toxin subcutaneously and sodium ricinoleate injected intravenously twenty-four hours later in doses ranging from 0.003 to 0.030 gm. per kilo. In other experiments the sodium ricinoleate was given intramuscularly in doses ranging from 0.1 to 0.5 gm. per kilogram, immediately after the subcutaneous injection of the toxin.

As shown in Table IV the untreated controls succumbed in forty-eight hours after the injection of toxin, as the dose was rather large but purposely selected in order to leave no doubt about the results. But unfortunately the sodium ricinoleate in the doses administered was unsuccessful in every instance in combating the toxemia, although the larger doses sometimes prolonged the lives of the animals by one or two days.

Duplicate tests embracing the intraperitoneal injection of sodium ricinoleate in doses ranging from 0.010 to 0.050 gm. per kilogram four hours after

TABLE IV  
THE EFFECT OF SODIUM RICINOLEATE UPON DIPHTHERIA TOXIN IN VIVO

| DIPHTHERIA TOXIN*<br>(1 c.c.) | SODIUM RICINOLEATE |                  | RESULTS IN DAYS |   |   |   |   |   |
|-------------------------------|--------------------|------------------|-----------------|---|---|---|---|---|
|                               | DOSE PER<br>KILO   | ADMINISTRATION   | 1               | 2 | 3 | 4 | 5 | 6 |
| 6 M. L. D.                    | 0.003              | Intravenously**  | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.006              | Intravenously**  | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.0075             | Intravenously**  | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.010              | Intravenously**  | -               | - | D |   |   |   |
| 6 M. L. D.                    | 0.015              | Intravenously**  | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.030              | Intravenously**  | -               | - | - | D |   |   |
| 6 M. L. D.                    | 0.1                | Intramuscularly† | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.15               | Intramuscularly† | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.2                | Intramuscularly† | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.3                | Intramuscularly† | -               | - | D |   |   |   |
| 6 M. L. D.                    | 0.4                | Intramuscularly† | -               | D |   |   |   |   |
| 6 M. L. D.                    | 0.5                | Intramuscularly† | -               | - | D |   |   |   |
| 3 M. L. D.                    | 0.010              | Intraperitoneal‡ | -               | - | - | D |   |   |
| 3 M. L. D.                    | 0.020              | Intraperitoneal‡ | -               | - | - | - | D |   |
| 3 M. L. D.                    | 0.040              | Intraperitoneal‡ | -               | - | - | D |   |   |
| 3 M. L. D.                    | 0.050              | Intraperitoneal‡ | -               | - | - | D |   |   |
| Control (6 M. L. D.)          | 0                  | 0                | -               | D |   |   |   |   |
| Control (6 M. L. D.)          | 0                  | 0                | -               | D |   |   |   |   |
| Control (6 M. L. D.)          | 0                  | 0                | -               | D |   |   |   |   |
| Control (6 M. L. D.)          | 0                  | 0                | -               | D |   |   |   |   |
| Control (3 M. L. D.)          | 0                  | 0                | -               | - | - | D |   |   |
| Control (3 M. L. D.)          | 0                  | 0                | -               | - | - | D |   |   |
| Control (3 M. L. D.)          | 0                  | 0                | -               | - | - | D |   |   |

\*By subcutaneous injection.

†Immediately after injection of toxin.

\*\*Twenty-four hours after injection of toxin.

‡- = survived; D = died.

§Given four hours after subcutaneous injection of toxin and repeated daily for 3 more doses.

the subcutaneous injection of 3 M. L. D.'s of toxin and repeated daily for 3 additional doses gave similar negative results, and as the experiments were repeated several times, it would appear that the compound is incapable of appreciably detoxifying diphtheria toxin in the living animal.

#### THE EFFECT OF SODIUM RICINOLEATE UPON TETANUS TOXIN

Similar results were observed with tetanus toxin except to state that in the test tube, sodium ricinoleate appears capable of detoxifying this toxin to a somewhat greater degree than diphtheria toxin.

For example an L+ dose of tetanus toxin in 1 c.c. of saline solution is apparently completely detoxified by 0.006 gm. of sodium ricinoleate dissolved in 1 c.c. of saline solution in an hour at room temperature as determined by injecting the mixtures subcutaneously into young guinea pigs (Table V).

Even a dose as small as 0.003 gm. detoxified this amount of toxin sufficiently to permit the animals to survive several days after the death of controls injected with the toxin alone.

Unfortunately, however, this inactivated toxin does not appear to possess antigenic value if one may judge from the results of a single experiment in which 5 L+ doses of toxin were added to 0.1 gm. of sodium ricinoleate dissolved in 50 c.c. of saline and incubated for an hour at 37° C. followed by

preservation in a refrigerator. Twelve guinea pigs were given 1 c.c. subcutaneously every five days for 10 doses. One animal succumbed before the series of injections were completed. Three weeks after the last dose the remaining

TABLE V

THE EFFECT OF SODIUM RICINOLEATE UPON TETANUS TOXIN IN VITRO

| TETANUS<br>TOXIN<br>(1 C.C.) | SODIUM<br>RICINO-<br>LEATE<br>(GM.) | RESULTS IN DAYS |   |   |   |   |   |   |   |   |    |    |
|------------------------------|-------------------------------------|-----------------|---|---|---|---|---|---|---|---|----|----|
|                              |                                     | 1               | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 12 |
| 1 L+ dose                    | 0.003                               | *               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.003                               | -               | - | - | - | D | - | D | - | - | -  | -  |
| 1 L+ dose                    | 0.006                               | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.006                               | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.0075                              | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.0075                              | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.01                                | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.01                                | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.015                               | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.015                               | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.030                               | -               | - | - | - | - | - | - | - | - | -  | -  |
| 1 L+ dose                    | 0.030                               | -               | - | - | - | - | - | - | - | - | -  | -  |
| Control                      | 0                                   | -               | D | - | - | - | - | - | - | - | -  | -  |
| Control                      | 0                                   | -               | D | - | - | - | - | - | - | - | -  | -  |
| Control                      | 0                                   | -               | D | - | - | - | - | - | - | - | -  | -  |
| Control                      | 0                                   | -               | D | - | - | - | - | - | - | - | -  | -  |

\* - = survived: D = died.

11 were given a subcutaneous injection of one-one-hundred-fiftieth of an L+ dose of toxin sufficient to cause the death of 4 controls on the third day. All of the immunized animals died in from five to eleven days, indicating that some immunity had been produced, but likewise, suggesting that sodium ricinoleate tends to de-antigenize a portion of this toxin as was probably also true of diphtheria toxin previously described.

But it would appear that sodium ricinoleate is capable of detoxifying tetanus toxin to some slight extent in the living animal.

Controls injected subcutaneously with one L+ dose of toxin died of tetanus intoxication in about forty-eight hours, whereas when 0.5 gm. of sodium ricinoleate was given intramuscularly, immediately after the toxin, some animals lived for one or two days longer (Table VI).

Controls injected subcutaneously with one-one-hundredth of the L+ dose died of tetanus in about three or four days, whereas the intramuscular injection of sodium ricinoleate in doses of 0.1 to 0.5 gm. per kilo immediately after the injection of toxin, prolonged the lives of some animals by one or two days.

Intravenous injections of 0.003 to 0.030 gm. per kilo twenty-four hours after the injection of toxin had no appreciable effect and the same was generally true of intraperitoneal injections. In the latter experiments guinea pigs were given subcutaneous injections of one-one-hundredth of the L+ dose of toxin sufficient to produce fatal tetanus intoxication in from three to four days. Four hours after the injection of toxin sodium ricinoleate was given intraperitoneally in doses varying from 0.010 to 0.040 gm. per kilo and repeated daily for 4 addi-

tional doses. All of the animals, however, developed tetanus and died in from four to six days, living, therefore, but a few days beyond the controls (Table VI).

Additional guinea pigs were given subcutaneous injections of one-one-hundredth of the L- dose of toxin and four days later when symptoms of tetanus first appeared were given 0.003 gm. of sodium ricinoleate intraspinally. This dose was repeated daily for 2 additional doses but all of the animals died in five or six days while the untreated controls succumbed in three or four days (Table VI).

TABLE VI

THE EFFECT OF SODIUM RICINOLEATE UPON TETANUS TOXIN IN VIVO

| TETANUS TOXIN*<br>(1 C.C.) | SODIUM RICINOLEATE |                   | RESULTS IN DAYS |   |   |   |   |   |
|----------------------------|--------------------|-------------------|-----------------|---|---|---|---|---|
|                            | DOSE PER<br>KILO   | ADMINISTRATION    | 1               | 2 | 3 | 4 | 5 | 6 |
| 1 L + dose                 | 0.1                | Intramuscular**   | -†              | D |   |   |   |   |
| 1 L + dose                 | 0.1                | Intramuscular**   | -               | D |   |   |   |   |
| 1 L + dose                 | 0.25               | Intramuscular**   | -               | D |   |   |   |   |
| 1 L + dose                 | 0.25               | Intramuscular**   | -               | D |   |   |   |   |
| 1 L + dose                 | 0.5                | Intramuscular**   | -               | - | D |   |   |   |
| 1 L + dose                 | 0.5                | Intramuscular**   | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.003              | Intravenously†    | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.006              | Intravenously†    | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.075              | Intravenously†    | -               | - | - | - | D |   |
| 1/100 of L + dose          | 0.010              | Intravenously†    | -               | - | - | - | - |   |
| 1/100 of L + dose          | 0.015              | Intravenously†    | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.030              | Intravenously†    | -               | - | D |   |   |   |
| 1/100 of L + dose          | 0.010              | Intraperitoneally | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.015              | Intraperitoneally | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.020              | Intraperitoneally | -               | - | - | - | D |   |
| 1/100 of L + dose          | 0.030              | Intraperitoneally | -               | - | - | - | D |   |
| 1/100 of L + dose          | 0.040              | Intraperitoneally | -               | - | - | - | - | D |
| 1/100 of L + dose          | 0.1                | Intramuscularly   | -               | - | - | - | - |   |
| 1/100 of L + dose          | 0.2                | Intramuscularly   | -               | - | - | - | - |   |
| 1/100 of L + dose          | 0.3                | Intramuscularly   | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.4                | Intramuscularly   | -               | - | - | D |   |   |
| 1/100 of L + dose          | 0.5                | Intramuscularly   | -               | - | - | - | - | D |
| 1/100 of L + dose          | 0.003              | Intraspinally     | -               | - | D |   |   | D |
| 1/100 of L + dose          | 0.003              | Intraspinally     | -               | - | - | - | D |   |
| 1/100 of L + dose          | 0.003              | Intraspinally     | -               | - | - | - | - | D |
| 1/100 of L + dose          | 0.003              | Intraspinally     | -               | - | - | - | - | D |
| Control (1 L +)            | 0                  | 0                 | -               | D |   |   |   |   |
| Control (1 L +)            | 0                  | 0                 | -               | D |   |   |   |   |
| Control (1/100 L +)        | 0                  | 0                 | -               | - | D |   |   |   |
| Control (1/100 L +)        | 0                  | 0                 | -               | - | - | D |   |   |
| Control (1/100 L +)        | 0                  | 0                 | -               | - | D |   |   |   |
| Control (1/100 L +)        | 0                  | 0                 | -               | - | D |   |   |   |

\*By subcutaneous injection. \*\*Immediately after injection of toxin.

†Twenty-four hours after injection of toxin. ‡- = survived; D = died.

These experiments repeated several times have shown, therefore, that sodium ricinoleate may have a slight degree of detoxifying activity for tetanus toxin in vivo; the doses of toxin were relatively large in order to produce death from tetanus of all of the controls which may have masked more evidence of detoxification in these experiments.

#### THE BACTERICIDAL ACTIVITY OF SODIUM RICINOLEATE

Sodium ricinoleate has a low bactericidal activity in the test tube.

When tested according to the Reddish method against *Staphylococcus pyogenes aureus*, it was found that whereas a 20 per cent solution was completely

bactericidal for this organism in an exposure of five minutes, yet 10 and 5 per cent solutions were not completely bactericidal in exposures as long as one hour. These results correspond closely to those of Gerwe<sup>9</sup> who also found the phenol coefficient for *B. typhosus* very low.

By the bacteriostatic method of Kolmer,<sup>10</sup> however, we have found that 1:4,000 concentrations of sodium ricinoleate inhibited the growth of *Staphylococcus aureus* in broth medium while 1:600 dilutions were bactericidal. With *Streptococcus hemolyticus* dilutions of 1:60,000 were bacteriostatic and 1:40,000 bactericidal; with *Pneumococcus* (Type I) dilutions up to 1:80,000 were bacteriostatic and 1:60,000 bactericidal.

#### SODIUM RICINOLEATE IN THE TREATMENT OF VARIOUS EXPERIMENTAL INFECTIONS

*In Streptococcus Cellulitis and Peritonitis of Mice.*—White mice were given intraperitoneal injections of 0.6 c.c. of twenty-four-hour broth cultures of *Streptococcus hemolyticus* and four hours later intraperitoneal injections of sodium ricinoleate in doses varying from 0.050 to 0.250 gm. per kilogram of weight.

The culture was of such virulence that the 8 untreated controls died of the resulting peritonitis and septicemia in about forty-eight hours, all animals showing positive heart blood cultures at necropsy.

All of 20 mice receiving the compound died, however, in the same period of time so that single doses of sodium ricinoleate of the amounts employed were without appreciable effect. All of these mice, likewise, showed positive heart blood cultures.

Fourteen additional mice were infected with the same dose of culture by injection under the skin of the thorax. Four untreated controls died in from three to five days of the resulting cellulitis and with positive heart blood cultures obtained at autopsy.

Ten mice were given intraperitoneal injections of single doses of sodium ricinoleate, likewise varying from 0.050 to 0.250 gm. per kilogram of weight, but all of these also perished in about the same period of time as the untreated controls from the local abscesses associated with the septicemia (positive heart blood cultures).

From these experiments it appeared that sodium ricinoleate by intraperitoneal injection was without appreciable curative effects in streptococcus cellulitis and peritonitis of mice. It is true, however, that the infections were very severe but unfortunately this is a difficulty in the chemotherapy of experimental streptococcus infections of mice in that smaller doses of the organism permits some untreated controls to live indefinitely and therefore making it difficult to evaluate the results of treatment. On the other hand the rapidly fatal experimental infections have no parallel in streptococcus infections of the human being and may readily enough mask some degree of curative activity on the part of the compound under study.

*In Pneumococcus Peritonitis and Septicemia of Mice.*—Similar negative results were observed in the treatment of mice infected with a highly virulent culture of Type I pneumococcus.



Eight mice injected intraperitoneally with 0.3 c.c. of a 1:40,000 dilution of twenty-four-hour broth culture died in about forty-eight hours with positive heart blood cultures (septicemia). Twenty additional mice infected at the same time were given single doses of sodium ricinoleate intraperitoneally four hours later in doses varying from 0.006 to 0.050 gm. per kilogram of weight. All of these, likewise, succumbed in about the same time as the untreated controls, so that in this single experiment there was no evidence of curative activity on the part of the compound administered by intraperitoneal injection.

*In Tuberculosis of Guinea Pigs.*—A series of 24 guinea pigs weighing from 280 to 400 gm. were given subcutaneous injections in the neighborhood of the inguinal glands of 0.005 gm. of human tubercle bacilli. Four untreated controls died of the resulting infection in from twelve to eighteen days.

One week after infection 20 of the animals were given intraperitoneal injections of sodium ricinoleate in doses varying from 0.030 to 0.100 gm. per kilo of weight and the injections repeated every three days. Two of the animals receiving the 0.100 gm. dose died after three injections from the toxicity of the drug and before tuberculous lesions had well developed. From 2 to 8 injections of sodium ricinoleate were given the 18 remaining animals, but all died of widespread tuberculous infection in from eight to twenty days, so that the compound was without appreciable curative effects. Indeed a number of the animals receiving the larger doses of sodium ricinoleate died a few days earlier than the untreated controls and suggesting that their resistance to tuberculous infection had been reduced by the compound.

*In Syphilis of Rabbits.*—Eight rabbits were given intratesticular inoculations with our Nichols-Hough strain of *Spirocheta pallida*. One month later two untreated controls developed acute bilateral orchitis with numerous spirochetes in dark-field examinations.

One week after testicular inoculation 6 of the rabbits were given 0.003 gm. of sodium ricinoleate per kilogram of weight by intravenous injection, and the dose was repeated daily for a total of 8 injections.

All of these animals likewise developed acute testicular syphilis with positive dark-field examinations in about a month, so that the compound was without any demonstrable curative effects in this single experiment.

*In Trypanosomiasis of Rats.*—White rats weighing from 110 to 300 gm. were given intraperitoneal injections of approximately 500,000 *Tryp. equiperdum*. Four untreated controls died of the resulting infection in from three to four days and showed enormous numbers of the trypanosomes in the tail blood.

Twenty-four hours after infection and before parasites appeared in the peripheral blood, 20 of the rats were given single intravenous injections of sodium ricinoleate in doses varying from 0.005 to 0.025 gm. per kilogram of weight. All of these likewise succumbed to trypanosomiasis in from three to four days and showed enormous numbers of parasites in the peripheral blood, so that the compound was without appreciable trypanocidal activity in this single experiment.

*In Acute Poliomyelitis of Monkeys.*—Three monkeys were inoculated intracerebrally with an emulsion of monkey poliomyelitic spinal cord kindly fur-

nished by the Rockefeller Institute. The first clinical evidences of poliomyelitis was apparent in from seven to nine days, and an untreated control died of paralysis twenty-one days after inoculation.

The two remaining animals were given sodium ricinoleate in dose of 0.005 gm. per kilogram on the seventh day after inoculation when signs of infection were first apparent and the dose repeated daily for a total of five injections. Both animals died in from eighteen to twenty-two days from progressive paralysis, so that sodium ricinoleate was without demonstrable curative effects in these two animals, a more complete description of the experiment being given in a separate communication.<sup>11</sup>

#### SUMMARY

1. Sodium ricinoleate (sorcin) is compound of particular interest from the chemotherapeutic standpoint because of the possibility of inactivating toxins and other bacterial agents in the blood.

2. Its toxicity has been determined by intravenous, intraperitoneal, intramuscular and subtheal injection in the lower animals.

3. It is highly hemolytic and upon intravenous injection may produce intravascular hemolysis with embolism. The maximum tolerated dose by this route of administration has been found to be approximately 0.035 gm. per kilo.

4. By intramuscular injection it may produce local irritation with liquefaction necrosis when large amounts are injected. However it is well borne systematically by this route of administration as the maximum tolerated dose has been found to be more than 1 gm. per kilo.

5. It may also produce some irritation upon intraperitoneal injection but is well borne by this route, since the maximum tolerated dose is as high as 0.16 to 0.3 gm. per kilo.

6. By intracisternal injection the maximum tolerated dose has been found to be approximately 0.004 gm. per kilo.

7. Sodium ricinoleate is capable of inactivating or destroying diphtheria and tetanus toxins in the test tube and is especially destructive for the latter, although it would appear that the resulting toxoids are of low antigenic activity.

8. Sodium ricinoleate is but feebly antitoxic for the toxins of diphtheria and tetanus bacilli in guinea pigs by various routes of administration.

9. Sodium ricinoleate is of very low bactericidal activity in the test tube and has been found without demonstrable curative activity in severe streptococcal, pneumococcal, and tuberculous infections of the lower animals as well as in experimental trypanosomiasis of rats, syphilis of rabbits, and acute anterior poliomyelitis of monkeys.

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## THE SPECIFIC GRAVITY OF THE BLOOD IN HUMAN CANCER\*

### FOUR HUNDRED OBSERVATIONS, WITH A NOTE ON ITS CLINICAL SIGNIFICANCE

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**NORMAL Values.**—The normal specific gravity of the whole blood has been fairly consistently found to be about 1.055 for males and 1.053 for females (Jones,<sup>1</sup> Schmaltz,<sup>2</sup> Lyonnet,<sup>3</sup> Leake, Kohl, and Stebbins,<sup>4</sup> Polowe<sup>5</sup>). A diurnal variation was noted by Jones, Leake et al., and Polowe, the specific gravity of the whole blood being about 0.002 higher in the morning than in the afternoon. For the purposes of this study specific gravity values below 1.050 were considered as representing a state of anemia, while values above 1.060 were considered as representing a higher than normal density of the whole blood.

**Literature.**—No large series of observations on the specific gravity of the whole blood in human cancer has been made in recent years. Lloyd Jones, in 1887, reported a series of 15 cases. His lowest blood density was 1.031 obtained in a case of carcinoma of the uterus. But even in this small series he called attention to the fact that normal specific gravity values may be found in advanced cases of cancer. Schmaltz, in 1891, reported one case of carcinoma of the stomach in which a specific gravity of the blood of 1.039 was found. Polowe,<sup>6</sup> in 1929, reported his findings in 23 cases of proved carcinoma. He stated at that time that, "the more severe anemias occurred in carcinoma of hidden or not readily accessible areas (gastrointestinal tract, peritoneum, pancreas, urinary bladder). Carcinoma of other parts of the body did not seem to alter the normal blood density, even though most of the cases were quite advanced."

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*The Clinical Significance of the Specific Gravity of the Blood in Human Cancer.*—(a) Any discussion of the specific gravity of the blood presupposes the idea that a change in the quality of the blood is at once manifested by a change in its specific gravity. Such a change may be brought about by an alteration in the number of erythrocytes, leucocytes, platelets; by alteration of the hemoglobin content, fibrinogen content, ash content; by alteration of the lipid values; and by shifting water balances. In anemia associated with cancer\* (as in the anemia associated with other clinical entities) no one factor alone may be incriminated as the sole agent which so materially affects the weight of the blood.

Lucas,<sup>7</sup> in a very recent publication on the diagnosis and treatment of the anemias of cancer, states that if one searches diligently enough the cause of such anemias can usually be found and treated. He states further: "Anemia obviously may result from an increased destruction or loss of blood, from a decreased blood formation, or combination of these two. Decreased blood formation frequently is preceded by a chronic blood loss or destruction. Chronic and acute hemorrhage is usually not difficult to find so that we are chiefly concerned with decreased blood formation due to nutritional deficiency or to the inability of the patient to assimilate food properly." Lucas mentions the following known values to aid in determining the hematopoietic needs of the patient:

1. Red blood cell count
2. The percentage hemoglobin
3. The mean corpuscular volume
4. The color index
5. The free HCl of the gastric contents
6. The saturation index

I have quoted Lucas rather fully because I feel that for the average practitioner the specific gravity of the whole blood offers a rapid orientation of the status of the blood at any given moment and includes at least three (1, 2, and 3 above) of the needed values, and possibly five (the saturation index and, in cases of secondary anemia, the color index). Interpolations of specific gravity values into the above values may be made roughly by experience with the method or quite accurately by the use of Guthrie's comparison chart.<sup>8</sup> One should not forget the use of one's clinical judgment in evaluating any of these findings, including the specific gravity of the blood.

Factors that tend to increase the blood density should also be kept in mind when evaluating the blood status in any given case. An absolute increase in blood density may be brought about by an increase in the production of any of its various constituents. Examples of this may be found in polycythemia vera wherein the erythrocytes are markedly increased in number, though one should note that in this condition the percentage hemoglobin is relatively low; in hyperparathyroidism the increased calcium content of the blood increases the blood density. A relative increase in blood density may be brought about by dehydration due either to lack of fluid intake, lack of fluid intake plus anuria (Popel's<sup>9</sup> work on dogs), or to loss of fluids by excessive diaphoresis, emesis,

\*The term "anemia of cancer" should perhaps be avoided. The term "anemia associated with cancer" is suggested to emphasize the clinical fact that the anemia associated with cancer is almost never due to the cancer process itself.

diarrhea, or combinations of these. A relative increase in blood density may also be brought about by venostasis (Copeman's<sup>10</sup> work on rabbits) due to cardio-respiratory diseases or due to actual mechanical obstruction of the venous return by masses in the axilla, neck, thorax, and abdomen as may sometimes be seen in advanced cases of neoplastic disease. In the clinical evaluation of the blood status of the dehydrated carcinomatous patient I have found that the relatively high hemoglobin percentages found are often belied by low specific gravity values of the whole blood, the latter values being more in keeping with the clinical appearance of the patient. Examples of this may be found in cases of obstructive lesions of the stomach wherein vomiting (with due respect paid to starvation effects) causes not only a loss of water but of minerals, such as chlorides, as well. This produces a mineral imbalance between the blood stream and the tissue fluids which is more truly, though not ideally, reflected by the specific gravity of the whole blood than by the estimation of the now concentrated erythrocyte and hemoglobin values. An emotional anhydremia in needle-shy patients has been reported by Barbour and Hamilton.<sup>11</sup>

(b) The following is a study of the blood status associated with human cancer in terms of the specific gravity of the whole blood. The falling drop technic of Barbour and Hamilton<sup>12</sup> was used. The blood was obtained from the capillary bed at the finger tip. In lesions of the breast, axillae, and upper extremities, where only one side was involved, the finger on the sound side was selected to obviate as much as possible the venostatic effect of mechanical pressure.

Single observations were made on 400 cases of proved cancer (Table I) and, as a control on 50 cases of proved benign neoplasms and noncancerous lesions (Table II). Table III represents a further control group of 50 cases, in summary form, of average run, nonneoplastic conditions as found in a general hospital, the details of which were published elsewhere.<sup>3, 6</sup> Tables IV, V,

TABLE I  
THE SPECIFIC GRAVITY OF THE WHOLE BLOOD  
(COMPOSITE SUMMARY OF FINDINGS IN 400 CASES OF HUMAN CANCER)

| CLINIC              | NO. OBS. | SP. GR.<br>BELOW 1.050 | PER CENT<br>ANEMIC | SP. GR. 1.060<br>AND OVER | PER CENT<br>WITH<br>HIGH SP. GR. |
|---------------------|----------|------------------------|--------------------|---------------------------|----------------------------------|
| A. Breast           | 100      | 3                      | 3                  | 13                        | 13                               |
| B. Epitheliomas     | 23       | 1                      | 4                  | 5                         | 22                               |
| C. Sarcomas, neur.  | 50       | 6                      | 12                 | 9                         | 18                               |
| D. Rectal           | 100      | 20                     | 20                 | 1                         | 1                                |
| E. Gynecologic      | 50       | 8                      | 16                 | 3                         | 6                                |
| F. Oral             | 26       | 3                      | 12                 | 1                         | 4                                |
| G. Gastric          | 20       | 12                     | 60                 | 0                         | 0                                |
| H. Sarcomas, osteo. | 15       | 3                      | 20                 | 0                         | 0                                |
| I. Urologic         | 16       | 8                      | 50                 | 0                         | 0                                |
| Corrected           | 400      | 64                     | 16                 | 32                        | 8                                |
|                     | 900      | 197                    | 22                 | 64                        | 7                                |

and VI, respectively, represent the severe, the moderately severe, and the mild cases of the anemias associated with cancer as found in this study. The interpolations of the specific gravity values, as shown in Tables IV, V, and VI, into

hemoglobin content, red blood cell counts, and sediment volume percentage, were made from the comparison chart of Guthrie.<sup>8</sup> Notes (summarized in Table VII) were made as to the grades of malignancy and as to the pathologists' estimate

TABLE II  
THE SPECIFIC GRAVITY OF THE WHOLE BLOOD  
(COMPOSITE SUMMARY OF FINDINGS IN 50 CASES OF NONCANCEROUS LESIONS)

| CLINIC          | NO. OBS.  | SP. GR.<br>BELOW 1.050 | PER CENT<br>ANEMIC | SP. GR. 1.060<br>AND OVER | PER CENT<br>WITH<br>HIGH SP. GR. |
|-----------------|-----------|------------------------|--------------------|---------------------------|----------------------------------|
| B. Epitheliomas | 13        | 1                      | 8                  | 0                         | 0                                |
| E. Gynecologic  | 18        | 2                      | 11                 | 1                         | 5                                |
| G. Gastric      | 14        | 4                      | 30                 | 0                         | 0                                |
| H. Bone         | 2         | 0                      | 0                  | 1                         | 50                               |
| I. Urologic     | 3         | 3                      | 100                | 0                         | 0                                |
| Corrected       | 50<br>500 | 10<br>149              | 20<br>30           | 2<br>55                   | 4<br>11                          |

TABLE III  
THE SPECIFIC GRAVITY OF THE WHOLE BLOOD\*  
(FINDINGS IN 50 MISCELLANEOUS CASES AS FOUND IN A GENERAL HOSPITAL)

| WARDS   | NO. OBS. | SP. GR.<br>BELOW 1.050 | PER CENT<br>ANEMIC | SP. GR. 1.060<br>AND OVER | PER CENT<br>WITH<br>HIGH SP. GR. |
|---------|----------|------------------------|--------------------|---------------------------|----------------------------------|
| Various | 50       | 26                     | 52                 | 1†                        | 2                                |

\*From the Barnert Memorial Hospital, Paterson, N. J. 5, 6

†Diagnosis: Bronchial asthma.

TABLE IV  
THE SEVERE ANEMIAS OF HUMAN CANCER: SP. GR. 1.030 TO 1.039. HEMOGLOBIN LESS THAN 39 PER CENT; RED BLOOD COUNT LESS THAN 2,000,000 CELLS; SEDIMENT VOLUME LESS THAN 19 PER CENT

| CLINIC | AGE | SEX | DIAGNOSIS                               | GRADE | R.R. | R.S. | SP. GR. BLOOD |
|--------|-----|-----|-----------------------------------------|-------|------|------|---------------|
| G-12   | 52  | M   | Ca. Stomach, perforated<br>6 days later |       |      |      | 1.031         |
| G-2    | 57  | M   | Ca. Stomach                             |       |      |      | 1.034         |
| G-6    | 39  | M   | Ca. Cardia of Stomach                   |       |      |      | 1.036         |
| G-14   | 55  | F   | Ca. Stomach                             |       |      |      | 1.038         |

TABLE V  
THE MODERATELY SEVERE ANEMIAS OF CANCER: SP. GR. 1.040 TO 1.044. HEMOGLOBIN 40 PER CENT TO 59 PER CENT; R.B.C. TWO TO THREE MILLION CELLS. SEDIMENT VOLUME 20 PER CENT TO 29 PER CENT

| CLINIC | AGE | SEX | DIAGNOSIS                             | GRADE | R.R. | R.S. | SP. GR. BLOOD |
|--------|-----|-----|---------------------------------------|-------|------|------|---------------|
| D-69   | 75  | F   | Adenoca. Rectum                       | III   | Yes  |      | 1.040         |
| E-49   | 47  | F   | Primary Advanced<br>Ca. Cervix        |       |      |      | 1.040         |
| H-15   | 59  | F   | Neurogenic Myxosarc. of back          | II    | Yes  |      | 1.041         |
| E-23   | 20  | F   | Rectovaginal Ca. (extensive)          | III   |      |      | 1.041         |
| C-2    | 53  | F   | Lipo-sarc. rt. deltoid.               |       |      |      | 1.041         |
| E-41   | 48  | F   | Squam. ca. Cervix                     | II    | Yes  |      | 1.042         |
| E-37   | 43  | F   | Ca. Cervix                            |       |      |      | 1.043         |
| E-28   | 56  | F   | Ca. Cervix Recurrent<br>postoperative |       |      |      | 1.044         |
| E-31   | 45  | F   | Ca. Cervix                            |       |      |      | 1.044         |
| D-9    | 67  | F   | Adenoca. Rectum                       | I     | Yes  |      | 1.044         |
| D-56   | 32  | F   | Adenoma malignum, Rect.               | II    |      |      | 1.044         |

TABLE VI

THE MILD ANEMIAS OF HUMAN CANCER: SP. GR. 1.015 TO 1.019. HEMOGLOBIN 60 PER CENT TO 79 PER CENT; R.B.C. THREE TO FOUR MILLION CELLS; SEDIMENT VOLUME 30 PER CENT TO 39 PER CENT

| CLINIC | AGE | SEX | DIAGNOSIS                                                            | GRADE    | R.R. | E.S. | SP. GR. BLOOD |
|--------|-----|-----|----------------------------------------------------------------------|----------|------|------|---------------|
| G-11   | 56  | M   | { Ca., Pancreas (Diabetic)<br>Cholecystogastrostomy 3 mo.<br>prior } |          |      |      | 1.015         |
| I- 3   | 24  | M   | Teratoma Testis:<br>Embryonal Ca.                                    |          |      |      | 1.015         |
| I- 5   | 38  | M   | Pap. Ep. Ca. Bladder                                                 | I        | Yes  |      | 1.015         |
| D-36   | 43  | M   | Aden. Malig. Rectal                                                  | II       | Yes  |      | 1.015         |
| A-27   | 62  | F   | Ca. Breast, Postop.                                                  |          |      |      | 1.016         |
| E-38   | 52  | F   | Pap. Ep. Ca. Cervix                                                  | II       |      | Yes  | 1.016         |
| D-31   | 38  | F   | Gelatinous Aden. Ca.                                                 | III & IV | Yes  |      | 1.016         |
| D-37   | 32  | M   | Malig. Polyp. or Pap. Ep. Ca.                                        | III      |      |      | 1.016         |
| F- 2   | 74  | M   | Ca. Mucosa Cheek                                                     |          |      |      | 1.016         |
| F- 5   | 15  | M   | Anaplastic Ca. Nasophar.                                             | III      |      | Yes  | 1.016         |
| I- 7   | 70  | M   | Recurrent Ca. Bladder<br>(prostatectomy, 2 yr. before)               |          |      |      | 1.016         |
| I-12   | 68  | M   | Ad. Ca. Prostate                                                     |          |      |      | 1.016         |
| D-50   | 58  | M   | Adenoma Malig. Rectal                                                | II       | Yes  |      | 1.017         |
| D-80   | 47  | M   | Adenoca. Rectum (infiltrated)                                        | III      | Yes  |      | 1.017         |
| D-92   | 55  | F   | Gelat. Ad. Ca. Rectum                                                | III      | Yes  |      | 1.017         |
| C-44   | 29  | M   | Recurrent Neuro. Sarc.                                               |          |      |      | 1.017         |
| G- 1   | 40  | M   | Ca. Stomach                                                          |          |      |      | 1.017         |
| G-18   | 71  | M   | Ca. Stomach                                                          |          |      |      | 1.017         |
| H- 4   | 50  | F   | Myxosarcoma rt. leg                                                  |          |      |      | 1.017         |
| I- 4   | 61  | M   | Ep. Ca. Bladder                                                      | III      |      | Yes  | 1.017         |
| I-11   | 68  | M   | Ad. Ca. Prostate                                                     | II       | Yes  |      | 1.017         |
| D-23   | 64  | M   | Ad. Malignum, Rectum<br>(infiltrated)                                | II       | Yes  |      | 1.018         |
| D-58   | 70  | M   | Ad. Malignum, Rectum                                                 | II       | Yes  |      | 1.018         |
| D-93   | 45  | M   | Ad. Malignum, Rectum<br>(infiltrating)                               | II       | Yes  |      | 1.018         |
| A-32   | 63  | F   | Ca. Rt. Breast and Ca. Corp.<br>Ut. Preop.                           |          |      |      | 1.018         |
| A-35   | 60  | F   | Ca. Breast, Preop. Radiated                                          |          |      |      | 1.018         |
| C-24   | 67  | M   | Recurrent Neur. Sarcoma                                              |          |      |      | 1.018         |
| G- 3   | 48  | M   | Ca. Stomach Adv. inoperable.<br>Cardia                               |          |      |      | 1.018         |
| G- 4   | 62  | M   | Ca. Stomach, Fundus                                                  |          |      |      | 1.018         |
| H- 8   | 11  | F   | Osteogenic. Sarc. Femur.                                             |          |      |      | 1.018         |
| I- 9   | 48  | M   | Ad. Ca. Prostate, w. Bone met.                                       |          |      |      | 1.018         |
| I-16   | 45  | M   | Teratoma Testis                                                      |          |      |      | 1.018         |
| B-72   | 63  | M   | Superficial Sq. Ca.                                                  | I        | Yes  |      | 1.019         |
| C-18   | 62  | M   | Spindle cell sarcoma                                                 |          |      |      | 1.019         |
| C-40   | 72  | F   | Fibrosarc. lower extrem.                                             |          |      |      | 1.019         |
| C-73   | 51  | M   | Neurosarcoma                                                         |          |      |      | 1.019         |
| D- 2   | 50  | F   | Pap. Ad. Ca. Rectum                                                  | III      | Yes  |      | 1.019         |
| D-19   | 73  | M   | Ad. Ca. Rectum                                                       | III      | Yes  |      | 1.019         |
| D-26   | 73  | M   | Ad. Malig. Rectum                                                    | II       | Yes  |      | 1.019         |
| D-32   | 44  | M   | Colloid Ca. Rectum                                                   | II       | Yes  |      | 1.019         |
| D-33   | 51  | M   | Ad. Malignum Rectum                                                  |          |      |      | 1.019         |
| D-91   | 66  | F   | Colloid Ca. Rectum (infiltrated)                                     | II       |      |      | 1.019         |
| D-95   | 60  | F   | Ad. Malignum, Rectum<br>(extensive with chr. inflam.)                | II       |      |      | 1.019         |
| D-97   | 46  | F   | Ad. Ca. Rectum                                                       | III      | Yes  |      | 1.019         |
| E-39   | 29  | F   | Ca. Cervix                                                           |          |      |      | 1.019         |
| F-28   | 62  | M   | Sq. Ca. Lip                                                          | I        | Yes  |      | 1.019         |
| G- 7   | 62  | F   | Ad. Ca. (Pancreas?) Cyst                                             |          |      |      | 1.019         |
| G-16   | 50  | M   | Ca. Stomach (with rt. sub-<br>phrenic abscess)                       | III      | Yes  |      | 1.019         |

of the radiosensitivity of the cancerous growths in the last 300 cases observed. Table VIII represents the cases of anemia found in the noneancerous group of lesions.

In the series of 23 cases I reported in 1929, ten, or 43 per cent, fell into the anemic group. This is in striking contrast to the present larger series of 400 observations where the corrected incidence of anemia was found to be 22 per cent. This may be explained not alone by the greater accuracy obtained in observing a larger series, but also by the fact that the smaller series was observed in hospitalized patients, usually late stages of cancer, whereas the larger series was compiled almost wholly (with the exception of about 6 cases) from ambulatory, out-patient cases. This low incidence of the anemias associated with early, ambulatory cases of cancer emphasizes the belief that cancer, at least in its beginnings, is a local disease. The 22 per cent incidence compares favorably with the 30 per cent incidence of anemias associated with neoplasms other than those of cancer; it contrasts strikingly with the 42 per cent of combined hospital and clinic patients (85 cases) of a general hospital as reported by Polowe,<sup>6</sup> and still more strikingly with the 52 per cent of purely hospital cases as noted in Table III.

This low incidence of anemia of 22 per cent bears further comment. It tends to minimize the current idea that irradiation of the cancerous growths produces anemia. I do not have the exact data as to how many of the observed cases were under radiation treatment, but I do recall that most of them had received some such treatment.\* Lucas<sup>7</sup> feels that this factor as a cause of anemia is overemphasized. Examination of Tables IV, V, and VI tends to bear this out. Only 1.4 per cent of the observations (Table I) fell into the group of severe anemias. All four were in cancer of the stomach. Only 3.8 per cent of the observations fell into the moderately severe group of anemias. The majority of these were associated with cancer of the cervix and of the rectum. The balance of 16.8 per cent fell into the mild group of anemias. The lesions encountered were mainly those of the gastrointestinal tract, and those of the genitourinary tract (urinary bladder, testis, cervix). All of these three groups of anemias can be plausibly explained on a clinical basis (the presence of large ulcerated areas from which there is a constant oozing of blood and through which bacteria gain easy access to the body). Starvation and dehydration are no mean factors in the cause of anemia associated with cancer of the gastrointestinal tract. Irradiation should not, therefore, be considered an important factor in the production of anemia.

Starvation, hemorrhage, and infection form the great etiologic triad in the production of the anemias associated with cancer. The nature of this study (i.e., the single observations and the lack of follow-up) prevents an accurate interpretation of the blood status case by case. However, the number observed, plus some clinical note on each case, plus clinicopathologic follow-ups on some of the cases enable me to give a fair cross-section of my clinical impressions as

\*My notes show that in the breast series 18 cases (18 per cent) were treated by some form of irradiation. Of these only one fell into the anemic group (mild, with specific gravity of 1.048). Sixteen had blood densities within normal limits. One had a blood density of higher than normal, 1.061, and had received treatment with radium tubes and high voltage cycle.



to the factors determining the variation of the percentage of anemias in the various clinics. Thus two main groups stand out: (a) Cancer of the easily visible portions of the body as found in the breast, skin, oral, and gynecologic clinics. It is plausible to assume that the percentage of anemias is less in this group since the lesions are discovered earlier by the patient and are, therefore, treated earlier. (b) Cancer of the hidden portions of the body as found in the gastrointestinal and urologic clinic. It is plausible to assume that the percentage of anemias is greater in this group as the patient usually discovers his condition when the growth has advanced and usually long after starvation, hemorrhage, and infection have made inroads into his body economy.\*

In the sarcomas attention is invited only to the fact that those involving bone (a blood-forming organ) exhibit at least half again a greater incidence of anemia than do those involving the nerves.

The group of higher than normal blood densities shown in the last two columns of Table I deserves comment. I cannot explain the high blood density in all the cases, but my clinical notes indicate that venestasis may be an important etiologic factor. Thus of the 13 breast cases one patient was noted as being inoperable, one was noted as having edema, and 7 were postoperative cases with possible interference of venous return, though my notes do not specifically show that in these 7 cases. In the group of 5 patients with epitheliomas, one patient with a specific gravity of 1.062 is noted as having a squamous carcinoma with metastasis to the axillary nodes, one patient with a specific gravity of 1.064 is noted as having a squamous carcinoma with clubbed finger tips, the other three patients are noted as having squamous carcinomas on hands and arms. In the neurogenic sarcoma group, of the 4, one involved the left forearm and the other a right lower extremity, but my notes do not indicate that there was involvement of the regional lymph nodes, which if present might account for an increased blood density. One melanosarcoma is noted as involving the right axilla. One extensive angiosarcoma of the back was associated with a specific gravity of the blood of 1.062.

In this group of higher than normal blood densities, it is the negative phase which compels attention. Thus, no high blood densities appear in any of the gastric cases. It is in this clinic that I saw cases of dehydration where one would expect a high blood density (especially in view of the normal or nearly normal red blood cell counts and hemoglobin values that were found). But such was not the case. Other factors, such as loss, by emesis, of ash content of the blood, and factors as yet undetermined tend to produce a blood density more in keeping with the clinical picture. The same is true of cancer of the large bowel where only one case (1 per cent) was found to have a blood density of 1.061.

It must be borne in mind that the complexities of the forces at play, such as the fluctuating water and mineral balances and the destroyed rhythm of blood formation, tend at times to produce normal specific gravity values while pathologic forces are destroying the patient. The importance of clinical judgment is obvious.

\*A striking example of this was seen in Case G-12, Table IV, carcinoma of the stomach in a male, aged fifty-two, wherein a blood density of 1.031 (the lowest density observed in the entire series) was found six days prior to perforation of the stomach.

Just a note in passing on the benign group of higher than normal blood densities. One case only deserves comment, that of a boy of nineteen, with a bone cyst, wherein the specific gravity of the blood was 1.061. This high blood density may have been due to an increased calcium content of the blood. The high specific gravity of the blood may be of differential diagnostic value in such cases.

(c) *Grading and Sensitivity.*—In Table VII one sees that 20 per cent of the cases graded fell into the anemic group, the more malignant grades (II and

TABLE VII  
GRADING AND SENSITIVITY REPORTS REVIEWED IN 300 CASES OF HUMAN CANCER

|  |                                        |     |
|--|----------------------------------------|-----|
|  | Total graded as to malignancy          | 148 |
|  | Total qualified as to radiosensitivity | 137 |
|  | Total qualified and graded             | 131 |
|  | Total graded but not qualified         | 17  |
|  | Total qualified but not graded         | 11  |
|  | Total neither graded nor qualified     | 152 |

| GRADE NONANEMIC: SP. GR. 1.050 OR OVER |          | ANEMIC: SP. GR. UNDER 1.050 |    |
|----------------------------------------|----------|-----------------------------|----|
|                                        | NO. OBS. | PER CENT                    |    |
| I                                      | 21       | 18                          | 4  |
| II                                     | 65       | 55                          | 14 |
| III                                    | 30       | 26                          | 12 |
| IV                                     | 1        | 1                           | —  |
|                                        | 117      | 80% of total graded         | 30 |
| R.R.                                   | 84       | 79%                         | 22 |
| R.S.                                   | 28       | 90%                         | 3  |

TABLE VIII  
THE TEN ANEMIAS AMONG THE 50 CASES OF HUMAN NONCANCEROUS LESIONS

| CLINIC | AGE | SEX | DIAGNOSIS                | SP. GR. BLOOD |
|--------|-----|-----|--------------------------|---------------|
| G- 6b  | 53  | M   | Achylia gastrica         | 1.043         |
| I- 3b  | 68  | M   | Hyp. prostate postop.    | 1.043         |
| I- 2b  | 66  | M   | Hyp. prostate postop.    | 1.044         |
| I- 1b  | 77  | F   | Epithelial papilloma     | 1.046         |
| E-13b  | 47  | F   | Fibroid uterus           | 1.047         |
| E-10b  | 28  | F   | Fibroid uterus           | 1.048         |
| G- 4b  | 53  | M   | Gastric ulcer            | 1.048         |
| G- 5b  | 55  | M   | Gastric ulcer            | 1.049         |
| G-11b  | 35  | M   | Mycosis fungoides (skin) | 1.049         |
| G-14b  | 78  | M   | Achylia gastrica         | 1.049         |

III) claiming the greatest number. This 20 per cent is in keeping with the 22 per cent incidence of the anemias in the 400 cases observed. Of the radio-resistant lesions 21 per cent fell into the anemic group. Here again the incidence is in keeping with the 22 per cent for the entire series. Of the radiosensitive lesions 10 per cent fell into the anemic group. No radiosensitive lesions were found in the severe and moderately severe groups of anemias (Tables IV and V). It appears quite likely that the low incidence of radiosensitive lesions among the anemias is due to their rapid growth and consequent early discovery by the patient who presents himself for treatment before anemia has set in.

From a statistical point of view, therefore, radioresistance is only coincidentally associated with an anemic status.

In 23 of the 31 lesions estimated as being radiosensitive, qualifying terms such as "relatively radiosensitive" or "moderately radiosensitive" were used. The estimate of sensitivity made by the pathologist appears to be ultimately conditioned by the size, location, and accessibility of the growth. Borak<sup>13,14</sup> terms these the conditional and constitutional factors, the conditional factors being determined by the character of the surrounding tissues, including regional and distant organs; the constitutional factors being determined by the nature of the tumor and of its matrix. It should be noted that Borak qualifies his conclusions by stating that, in addition to these biologic factors, the rôle of other clinical factors and the physical properties of the rays must be considered for prognosis and technic of irradiation. Pack and Quimby<sup>15</sup> state that radioresistance is a latent property of cancer tissue. They cite Canti as properly suggesting that "in the case of neoplasms there may be an indirect factor playing an important part." The nature of this indirect factor is not mentioned. Nemenow<sup>16</sup> feels that since the roentgen rays influence the metastructure of the cells, the molecules and atoms, it is as a rule impossible to estimate the radio-sensitivity on the basis of the histologic picture that reveals only the gross structure of the cell. To me, this seems like a hint that cellular chemistry may be a factor of influence.

Several men have thought it likely that the blood status influenced the sensitivity of the tumor process. Dean,<sup>17</sup> ten years ago, made the statement, "The relationship between our skin tests, the therapeutic results and the patients' blood quality has been so close as to warrant the prediction of the effect of therapy within certain limits on the basis of the latter factor alone." Dean found that the usual skin erythema dose had no effect on the anemic patient. After blood transfusion in such a case, Dean obtained a skin erythema with the usual dose. More recently, Stewart<sup>18</sup> very clearly presented the status of estimated sensitivity and observed results of irradiation of pathologic tissues. He stated, "The entire situation suggests the presence of some entirely unknown factor or factors influencing the sensitivity of the tumor process." Stewart further stated that, "After all, the only law of radiosensitivity which remains essentially unchallenged is that one accomplishes very little by irradiating a cachectic patient, unless the cachexia results from a highly sensitive tumor of the type of lymphosarcoma, thymoma, Wilms's tumor and the like." My own impression (stated here merely as a matter of record and as a subject for future investigation) is that there is some underlying electrolytic factor in the blood, whether the patient is anemic or not anemic (I have in mind a chloride retention), which in some way hinders the selective action of the rays. This impression is based on the work of Stammer and Moraczewski,<sup>19</sup> Macht and Teagraden,<sup>20</sup> and on some of my own unpublished work. This remains to be proved. I feel that the anemia is only coincidental, and that when it is associated with a radioresistant lesion, it (the anemia) is then only a clinical expression of a modified electrolytic content of the blood and should be so treated (as by transfusion) before irradiation of the patient.

## SUMMARY

1. The specific gravity of the whole blood was determined in 400 cases of proved cancer. A relatively low incidence of anemia was found: 22 per cent of the series as a whole; 5.2 per cent incidence of combined severe and moderately severe grades of anemia.

2. These anemias can usually be explained on a clinical basis as secondary effects of the cancer process and may so be treated.

3. Anemia appears to be only coincidentally associated with radioresistant lesions.

4. It appears that irradiation as a cause for anemia is overestimated.

5. Venestasis may be an important etiologic factor in the production of higher than normal blood densities.

6. The falling drop technic for determining the specific gravity of fluids offers an easy, rapid, and accurate orientation as to the status of the blood at any moment. It permits of limited differential diagnostic value in gastric lesions wherein low blood densities (expressive of severe or moderately severe grades of anemia) may make one lean toward a diagnosis of cancer.<sup>6</sup> In bone lesions, where hyperparathyroidism is suspected, higher than normal blood densities may add corroborative evidence.

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555 EAST TWENTY-SEVENTH STREET

# LABORATORY METHODS

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## DETERMINATION OF THE STROKE VOLUME OF THE HEART\*

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THERE is an occasional demand in routine laboratories to determine the stroke volume of the heart in connection with cardiac constriction cases. This may be obtained by determining the oxygen content of venous and arterial blood and the oxygen consumption of the lungs.

*Principle.*—The one-minute oxygen consumption of an individual at rest and fasting for several hours is determined using a basal metabolism apparatus. The pulse is recorded; the oxygen content of venous and arterial blood is determined. The stroke volume can then be calculated.

*Method.*—A. The oxygen consumption of the patient is determined by means of a basal metabolism apparatus. The temperature of the oxygen in the apparatus is noted, also the barometric pressure. The number of cubic centimeters of oxygen consumed per minute is converted to standard conditions by calculation.

B. About 5 c.c. of blood are withdrawn from a vein in the arm of the patient. The blood is protected from contact with air by using a Kaufman Luer double tip syringe, the side arm is connected by rubber tubing containing mineral oil to a test tube lined with approximately 25 mg. of sodium oxalate. A like amount of blood is withdrawn in a similar manner from an artery located in the wrist of the patient.

The pulse of the patient is recorded.

Sodium hydroxide solution: A 2 per cent solution of sodium hydroxide is aerated by introducing it into a separatory funnel and shaking it several minutes.

Oxygen reagent: Potassium ferrieyanide 3 gm., saponin 3 gm., caprylic alcohol 3 c.c., and distilled water, a sufficient quantity to make 1,000 c.c.

Using a van Slyke and Cullen CO<sub>2</sub> Pipette, 7.5 c.c. of the oxygen reagent are introduced into the cup of the apparatus and deaerated by subjecting it to repeated negative pressures. When the reagent is gas-free, 6 c.c. are forced up into the cup of the apparatus. Two cubic centimeters of venous blood are transferred from under the oil in the test tube to the cup of the CO<sub>2</sub> pipette, slowly being delivered under the reagent simultaneously being permitted to enter the chamber of the pipette followed by 5 c.c. of reagent. The excess of reagent is removed from the cup of the pipette. A few drops of mercury are introduced to act as a stopcock seal.

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\*From the Laboratories of the Graduate Hospital, University of Pennsylvania.  
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Most of the mercury is evacuated from the chamber of the pipette and the apparatus is shaken until the gases liberated from the blood by the reagent give a constant volume. (Shake from five to ten minutes.) The gases are oxygen, nitrogen, and carbon dioxide. A negative pressure is now produced in the chamber of the pipette by bringing the upper surface of the liquids to the 2 c.c. graduation. The cup of the pipette is rinsed with distilled water, and 0.5 c.c. of aerated sodium hydroxide is permitted to enter the chamber of the pipette followed by a few drops of mercury. One minute is allowed for the sodium hydroxide to combine with the carbon dioxide gas. The gases (oxygen and nitrogen) are now brought to atmospheric pressure and a reading taken. The temperature and barometric pressure are noted during the analysis.

C. The oxygen content of arterial blood is determined in the same way as that of venous blood.

#### Calculation.—

- A. A—c.c. of  $O_2$  consumed per minute (from basal metabolism apparatus)

$P_1$ —mm. barometric pressure

$P_2$ —760 mm.

$T_1$ —Absolute temperature of gas

$T_2$ —273 degrees Absolute

A.V.T. = Aqueous vapor tension

$$O_2 \text{ c.c. consumed per minute} = \frac{A \times T_2 \times (P_1 - A.V.T.)}{T_1 \times P_2}$$

- B.  $O_2$  in *venous blood*.

B = c.c. of  $O_2 + N_2$  in blood.

$$\text{Volume per cent } O_2 = \left[ \frac{P_1 - A.V.T.}{760 (1 + 0.00367 \times ^\circ C)} \times 50 \times B \right] - 1.36$$

1.36 = Volume per cent of  $N_2$

- C.  $O_2$  in *arterial blood*.

Use the same formula as for oxygen in venous blood.

- D. Minute volume of blood in cubic centimeters =  $\frac{100 \times \text{c.c. } O_2 \text{ consumed per minute}}{\% O_2 \text{ in "C" } - \% O_2 \text{ in "B"}}$

$$\text{Stroke volume in cubic centimeters} = \frac{\text{Minute volume of blood in c.c.}}{\text{Pulse}}$$

Normal stroke volume for patient at rest is from 50 to 100 c.c. in each ventricle per beat.

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## THE PREPARATION AND USE OF COLLOIDAL CARBON SOLUTIONS\*

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DURING an investigation undertaken a number of years ago, by the senior author, of the chemical changes in the spinal fluid responsible for the flocculation of the gold sol in the colloidal gold test, it was found that no correlation could be drawn between the two factors because of the variability of the gold sol.

It was found that two gold sols, even though prepared at the same time with the same reagents under the same conditions, did not give identical curves with the same spinal fluids. It was further found that one could not keep a satisfactory gold sol for a sufficiently long time to carry out a series of experiments which would extend over a period of months. In order to carry out the investigation as planned, it was necessary either to have a colloidal solution which could be made in large amounts that would keep indefinitely, or else to have one which could be made at different times and still yield identical results with spinal fluids.

As none of the colloidal solutions then being used could satisfactorily meet these requirements, a search was made for other colloids. After a number of solutions were investigated, it seemed that a colloidal suspension of carbon offered most promise of fulfilling the requirements. A preliminary report on the preparation and use of colloidal carbon solutions was presented at the meeting of the Federation of American Societies for Experimental Biology in 1927.<sup>1</sup> In 1928, at the Philadelphia meeting of the Interurban Clinical Club, a paper was presented giving results of further studies with the method. At this time it was pointed out that solutions of India ink would give similar results, although the reagent was not as sensitive as the electrolytic ones.

A further demonstration of this method was made at the International Physiological Congress at Boston in 1929. At that time a carbon solution made three years previously was used with spinal fluids obtained through the courtesy of Dr. Frank Fremont Smith of the Department of Neurology at Harvard Medical School. The carbon curves in every instance agreed with those obtained independently in his laboratory by the colloidal gold test. The curve of patient M. B. in Table VI was obtained with this solution. Recently Schube and Harms<sup>2</sup> have published an article confirming in part these findings, although no reference was given as to the original investigations of Looney.

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## METHOD

The preparation of colloidal solutions of carbon is readily accomplished by means of electrolysis between two carbon electrodes in a faintly acid medium. Solutions prepared by acid alone are as a rule not stable, but the addition of an oxidizing agent such as potassium dichromate increases their stability.

The carbon electrodes are obtained from discarded dry cells and it is preferable to use electrodes which have not been impregnated with paraffin. These are connected in series with a 22 ohm rheostat, capable of handling 5 amperes, an ammeter and the terminals of a 110 volt source of direct current. A volt meter is connected across the two electrodes which are immersed in a jar containing three liters of water to which has been added 3 ml. of concentrated sulphuric acid saturated with potassium dichromate. The desired amperage and voltage were obtained by regulating the distance between the electrodes and by manipulating the rheostat. Current was passed through the solution for from ten to twenty hours, yielding a black solution of carbon in colloidal suspension. After standing for several days the clear supernatant solution was decanted from the larger particles which had settled out. A good solution should be clear, dark brownish in color, and of such concentration that print can be distinguished through a layer 5 or 6 mm. in thickness. This depth of color can be obtained by diluting with distilled water. Pale solutions should be discarded.

When added to spinal fluids, varying degrees of precipitation may be observed and utilized as with colloidal gold solutions to yield a curve. The following scale has been used in recording observations:

- 0—no change
- 1—no precipitate, change in color
- 2—light precipitate, deep color
- 3—heavy precipitate, marked color
- 4—heavy precipitate, faint color
- 5—completely colorless

Eleven tubes were set up as for colloidal gold curves, 1 ml. of 1.0 per cent sodium chloride solution placed in each tube, and 1 ml. of spinal fluid added to the first tube. After thorough mixing 1 ml. was transferred from the first tube to the second, mixed thoroughly, and the process repeated to give a series of dilutions  $\frac{1}{2}$ ,  $\frac{1}{4}$ ,  $\frac{1}{8}$ , etc., the 1 ml. for transfer from the tenth tube being discarded, and the eleventh tube acting as control. Two ml. of the colloidal carbon solution were added to each tube, the racks shaken and allowed to stand overnight.

In Table I are given some of the preliminary experiments carried out during 1927 and 1928. It will be noted in Case 1 on March 15 that different carbon solutions give somewhat different results although the curves are of the same type. The character of the curve changed as the patient recovered so that it approached that of the normal in which precipitation occurs only in the first two tubes. That the curve obtained in meningitis differs in character from that obtained in general paresis is also evident from this table.

Although these cases showed that colloidal carbon solutions appeared to be suitable as a test medium, it was felt that a thorough study of all the factors influencing the sensitivity of the solutions was needed before the method could be used clinically.

The sensitivity of the reagent is influenced by the conditions of preparation and also by the salt concentration and  $P_H$  of the solution used to make the spinal fluid dilutions. Solutions were prepared using a current density of 2, 3, and 4 amperes at potentials of 20, 30, and 40 volts.

It was found that solutions prepared at the higher voltages were as a rule too unstable for use. Thus, of 7 samples made at 40 volts, 6 precipitated out completely after a few days' standing, and the seventh was so sensitive that the addition of 1 per cent salt solution caused complete precipitation. The samples prepared at very low voltages and amperages did not as a rule contain sufficient carbon to make them available for study. Stable solutions

TABLE I

| NO. | PATIENT | DATE | SAMPLE | CARBON CURVE | DIAGNOSIS                |
|-----|---------|------|--------|--------------|--------------------------|
| 1   | W. L.   | 3/ 8 | D      | 1155531000   | Meningitis               |
|     |         | 3/15 | D      | 1115551000   | Meningitis               |
|     |         | 3/15 | E      | 1145552000   | Meningitis               |
|     |         | 3/15 | G      | 1135554320   | Meningitis               |
|     |         | 3/20 | G      | 55430000000  | Meningitis               |
|     |         | 3/21 | G      | 54300000000  | Meningitis               |
| 2   | ----    | 3/19 | G      | 55540000000  | Meningitis               |
| 3   | J. H.   | 4/15 | G      | 2445544321   | Meningitis               |
|     |         | 4/18 | G      | 1155554332   | Meningitis               |
| 4   | T. W.   | 4/15 | G      | 000555432    | Meningitis               |
|     |         | 4/18 | G      | 000555543    | Meningitis               |
| 5   | C. S.   | 9/16 | D      | 1255544330   | Meningitis               |
|     |         | 9/16 | E      | 2345511000   | Meningitis               |
| 6   | M. B.   |      |        | 00235544320  | Tuberculous meningitis   |
| 7   | ----    | 3/25 | G      | 54000000000  | Maternity, normal        |
| 8   | ----    | 3/20 | G      | 54000000000  | Normal                   |
| 9   | S. H.   | 6/13 | D      | 21000000000  | Normal                   |
| 10  | F. P.   | 4/ 4 | G      | 55543210000  | Wassermann positive G.P. |
| 11  | ----    | 6/13 | D      | 5543211000   | Wassermann positive G.P. |
| 12  | J. N.   | 7/19 | D      | 5543200000   | Wassermann positive G.P. |
| 13  | J. H.   | 9/18 | D      | 5443222110   | Wassermann positive G.P. |

could be prepared at from 20 to 30 volts and from 3 to 4 amperes. The solutions made at 4 amperes were considerably more sensitive than those made at 3 amperes. The samples prepared with electrodes having a small surface area immersed in the solution were on the whole more sensitive than those having the same voltage and amperage but with a greater area of electrode in the bath. The best preparations are obtained with fresh electrodes immersed to a depth of from 13 to 14 cm. at 30 volts and 3 amperes. These solutions contain the maximum amount of carbon, about 250 to 270 mg. being contained in each 100 ml.

Variation in the sensitivity and stability of the solutions can be obtained by altering the salt concentration of the solution used in making the spinal fluid dilutions. This influence of the salt solution upon the sensitivity of the reaction is shown in Table II, which records the data when the dilutions were

made with distilled water, 1.0 per cent, and 1.5 per cent sodium chloride solutions. Increase in the salt concentration increases the sensitivity.

The stability of the carbon solutions on standing is a function of the sensitivity of the colloid. The less sensitive solutions can be kept for long periods, as is shown by the fact that the solution used for demonstration at the International Physiological Congress in 1929 had been prepared three years previously. On the other hand, extremely sensitive solutions have a tendency to precipitate out so that they are too light to use. Thus we find that Sample 6, when three weeks old, did not precipitate on the addition of two volumes of carbon to one volume of 2 per cent salt solution, but at six

TABLE II

| NO. | PATIENT | DATE | DILUTION  | CARBON CURVE |
|-----|---------|------|-----------|--------------|
| 13  | J. H.   | 9/18 | Water     | 5410000000   |
|     |         |      | 1.0% NaCl | 5443222110   |
| 14  | A. D.   | 9/18 | Water     | 5400000000   |
|     |         |      | 1.0% NaCl | 5442000000   |
| 15  | P. K.   |      | 1.5% NaCl | 4554321110   |
|     |         |      | 1.0% NaCl | 5542100000   |
| 16  | H. C.   |      | 1.5% NaCl | 5443211000   |
|     |         |      | 1.0% NaCl | 4431000000   |

months it was precipitated by 1.4 per cent salt but not by 1.2 per cent salt solution. This increase in sensitivity on standing is not sufficient in the case of the better solutions to prevent their being used for many months.

After the carbon has been added to the spinal fluid dilutions the readings can be made after standing overnight. Standing for a longer period has no effect on the curve obtained, as is shown by the readings taken at twenty-four hours and forty-eight hours in Table III.

TABLE III

| NO. | PATIENT | DATE  | HOURS | CARBON CURVE |
|-----|---------|-------|-------|--------------|
| 15  | P. K.   | 11/25 | 24    | 5542100000   |
|     |         |       | 48    | 5543100000   |
| 16  | H. C.   | 11/25 | 24    | 4431000000   |
|     |         |       | 48    | 4431000000   |

The solution as made is approximately 0.033 normal and is quite acid, various samples yielding  $P_H$  values by the quinhydrone electrode from 1.5 to 1.8. The influence of changes in acidity upon the sensitivity of the reaction was examined, the data of Table IV showing the influence of sodium carbonate and sodium hydroxide upon the precipitation of carbon by sodium chloride. The dilutions in Table IV were made by varying the concentration of sodium carbonate and sodium hydroxide in 2 per cent sodium chloride solution, using the 2:1 carbon: saline ratio as noted for the effect of salt concentration.

It will be seen that gradually increasing the amount of alkali added decreases the precipitation of the carbon solutions at first but finally the mixture again precipitates so that the addition of from 2 to 3 c.c. of 10 per cent

TABLE IV

| SAMPLE                                                                            | C.C. ADDED | 1.0 | 0.9 | 0.8 | 0.7 | 0.6 | 0.5 | 0.4 | 0.3 | 0.2 | 0.1 | 0.0 |
|-----------------------------------------------------------------------------------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A. Stabilizing Effect of Addition of 10 Per Cent NaOH to 2 Per Cent NaCl Solution |            |     |     |     |     |     |     |     |     |     |     |     |
| 6                                                                                 | 0          | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 2   | 2   | 3   |
| 23                                                                                | 2          | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 2   | 4   | 5   | 5   |
| 24                                                                                | 2          | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 4   | 5   | 5   | 5   |
| 26                                                                                | 0          | 0   | 0   | 0   | 0   | 0   | 1   | 3   | 5   | 5   | 5   | 5   |
| 27                                                                                | 2          | 2   | 2   | 0   | 0   | 0   | 0   | 2   | 3   | 4   | 5   | 5   |

| SAMPLE                                                                                                | C.C. ADDED | 1.50 | 1.35 | 1.20 | 1.05 | 0.90 | 0.75 | 0.60 | 0.45 | 0.30 | 0.00 |
|-------------------------------------------------------------------------------------------------------|------------|------|------|------|------|------|------|------|------|------|------|
| B. Stabilizing Effect of Addition of 10 Per Cent $\text{Na}_2\text{CO}_3$ to 2 Per Cent NaCl Solution |            |      |      |      |      |      |      |      |      |      |      |
| 6                                                                                                     | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 4    |
| 23                                                                                                    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 4    | 5    |
| 24                                                                                                    | 0          | 0    | 0    | 0    | 0    | 0    | 2    | 2    | 4    | 5    | 5    |
| 26                                                                                                    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 4    |
| 27                                                                                                    | 0          | 0    | 0    | 0    | 0    | 0    | 1    | 2    | 4    | 5    | 5    |
| 28                                                                                                    | 0          | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 1    | 1    |

sodium hydroxide causes complete precipitation. The zone in which there is no precipitation is much wider for sodium carbonate than for sodium hydroxide. This stabilizing effect of the addition of alkali can be utilized to adjust oversensitive carbon solutions, so that they can be made suitable for use with spinal fluids. This effect is given in Table V, in which the curves obtained by the addition of varying amounts of sodium carbonate to the colloidal carbon solutions are compared with the corresponding gold curve. The milliliters of 10 per cent sodium carbonate solution added to 100 ml. of colloidal carbon are recorded as the second value in the hyphenated sample number, e.g. 26-0.2 indicates that 0.2 ml. of 10 per cent sodium carbonate was

TABLE V

EFFECT OF ADDING 10 PER CENT  $\text{Na}_2\text{CO}_3$  ON THE CURVES OBTAINED FROM SPINAL FLUIDS

| SAMPLE AND<br>C.C. ADDED | CARBON CURVE | GOLD CURVE |
|--------------------------|--------------|------------|
| 26-0.0                   | 5544320000   | 4443210000 |
| 26-0.0                   | 4433220000   | 1110000000 |
| 26-0.2                   | 5421110000   | 5555310000 |
| 26-0.2                   | 5420000000   | 1111000000 |
| 26-0.5                   | 0000000000   | 0000000000 |
| 26-0.5                   | 0000000000   | 4332100000 |
| 6-0.0                    | 5542100000   | 5555353210 |
| 6-0.0                    | 4300000000   | 1111000000 |
| 28-0.0                   | 5542100000   | 5555432100 |
| 28-0.0                   | 5300000000   | 1111000000 |
| 28-0.5                   | 5400000000   | 4433210000 |
| 28-0.5                   | 4000000000   | 2222100000 |
| 23-1.0                   | 5554433200   | 5555532100 |
| 23-1.5                   | 555433322    | 5555532100 |
| 23-1.5                   | 5554432210   | 1111000000 |
| 23-1.8                   | 5432100000   | 5555310000 |
| 23-1.8                   | 4210000000   | 0000000000 |
| 23-2.0                   | 0000000000   | 5554210000 |
| 27-1.0                   | 5544333222   | 5544321000 |
| 27-1.8                   | 5532100000   | 5555532100 |
| 27-1.8                   | 2411000000   | 1111000000 |
| 27-2.0                   | 4322220000   | 5544321000 |

TABLE VI

| PATIENT              | DATE  | WASS.  | CELL   | GLOB. | SUGAR | PROT. | GOLD CURVE | CARBON CURVE           |
|----------------------|-------|--------|--------|-------|-------|-------|------------|------------------------|
| M.B.*                | 1929  | ----   | 110    |       | 24    | 269   | 0.511      | 0001123321 00235514320 |
| Carbon Sample 6      |       |        |        |       |       |       |            |                        |
| H.C.                 | 11/21 | pos.   | 8(RBD) | ST    | 112   | 65    | 0.714      | 5555555544 5532100000  |
| P.K.                 | 11/21 | pos.   | 155    | VST   | 77    | 105   | 0.708      | 555554321 5543200000   |
| L.G.                 | 11/14 | pos.   | 7(RBD) | T     | 62    | 74    | 0.588      | 5555353210 5542100000  |
| G.F.                 | 11/ 7 | pos.   | 4(RBD) | VST   | 75    | 59    | 0.628      | 4555533210 5443100000  |
| R.S.                 | 1/ 2  | pos.   | 7(RBD) | VST   | 79    | 51    | 0.719      | 4555521000 5544322100  |
| L.M.                 | 11/30 | pos.   | 4      | ST    | 46    | 53    | 0.717      | 4554321000 5543210000  |
| E.G.                 | 11/30 | pos.   | 8      | T     | 47    | 55    | 0.723      | 5553210000 5443210000  |
| A.A.                 | 11/30 | pos.   | 6      | ST    | 44    | 50    | 0.723      | 5532100000 5433200000  |
| M.G.                 | 1/ 2  | pos.   | 7(RBD) | VST   | 74    | 83    | 0.734      | 4444422100 5544333222  |
| A.C.                 | 11/30 | doubt. | 1      | VST   | 87    | 32    | 0.728      | 2221000000 4332100000  |
| F.B.                 | 11/14 | neg.   | 4      | SPT   | 66    | 33    | 0.602      | 1111110000 4300000000  |
| F.S.                 | 11/14 | neg.   | 1      | SPT   | 102   | 45    | 0.602      | 1111000000 5420000000  |
| R.D.                 | 11/14 | neg.   | 2      | VST   | 70    | 37    | 0.624      | 1111000000 4300000000  |
| A.C.                 | 11/14 | neg.   | 1(RBD) | SPT   | 77    | 34    | 0.620      | 0001110000 3100000000  |
| R.B.                 | 11/21 | neg.   | 6      | 0     | 109   | 44    | 0.713      | 0000000000 4200000000  |
| A.B.                 | 11/21 | neg.   | 4      | VST   | 110   | 35    | 0.712      | 0000000000 3000000000  |
| J.J.                 | 11/21 | neg.   | 5(RBD) | ST    | 110   | 30    | 0.722      | 0000000000 5300000000  |
| F.B.                 | 11/15 | neg.   | 3      | 0     | 54    | 43    | 0.625      | 0000000000 3200000000  |
| Carbon Sample 28     |       |        |        |       |       |       |            |                        |
| F.G.                 | 2/13  | pos.   | 2      | T     | 59    | 98    | 0.727      | 5555532100 5531110000  |
| H.S.                 | 2/20  | pos.   | 9      | ST    | 72    | 78    | 0.714      | 5555432100 5542100000  |
| R.G.                 | 2/27  |        | 3(RBD) | VST   | 72    | 47    | 0.743      | 5443210000 5421000000  |
| F.J.                 | 2/13  | pos.   | 1      | VST   | 65    | 56    | 0.727      | 4443210000 5322100000  |
| J.U.                 | 2/13  | pos.   | 2      | VST   | 68    | 53    | 0.722      | 3332100000 5320000000  |
| J.U.                 | 2/20  | doubt. | 1      | T     | 86    | 80    | 0.740      | 2222100000 5410000000  |
| C.C.                 | 2/20  | neg.   | 9      | VST   | 81    | 38    | 0.678      | 2221100000 4100000000  |
| J.V.                 | 2/13  | neg.   | 1      | ST    | 79    | 65    | 0.723      | 1111000000 5310000000  |
| R.S.                 | 2/20  | neg.   | 1      | VST   | 79    | 40    | 0.709      | 1111000000 3200000000  |
| J.G.                 | 2/20  | neg.   | 0      | SPT   | 68    | 48    | 0.722      | 1111000000 4200000000  |
| J.J.                 | 2/27  |        | 5(RBD) | ST    | 78    | 38    | 0.734      | 1111000000 5300000000  |
| A.H.                 | 2/13  | neg.   | 1      | SPT   | 53    | 30    | 0.702      | 1110000000 3100000000  |
| Carbon Sample 29     |       |        |        |       |       |       |            |                        |
| F.G.                 | 2/13  | pos.   | 2      | T     | 59    | 98    | 0.727      | 5555532100 5543220000  |
| H.S.                 | 2/20  | pos.   | 9      | ST    | 72    | 78    | 0.714      | 5555432100 5543321000  |
| J.U.                 | 2/20  | doubt. | 1      | T     | 86    | 80    | 0.740      | 2222100000 5432100000  |
| C.C.                 | 2/20  | neg.   | 9      | VST   | 81    | 38    | 0.678      | 2221000000 3221000000  |
| R.S.                 | 2/20  | neg.   | 1      | VST   | 79    | 40    | 0.709      | 1111000000 3320000000  |
| J.G.                 | 2/20  | neg.   | 0      | SPT   | 68    | 48    | 0.722      | 1111000000 4320000000  |
| Carbon Sample 27-2   |       |        |        |       |       |       |            |                        |
| A.P.                 | 2/ 7  | pos.   | 8      | VST   | 65    | 50    | 0.707      | 5544321000 4322220000  |
| F.J.                 | 2/13  | pos.   | 1      | VST   | 65    | 56    | 0.727      | 4443210000 4542100000  |
| J.U.                 | 2/13  | pos.   | 2      | VST   | 68    | 53    | 0.722      | 3332100000 5543322000  |
| A.P.                 | 2/ 7  | neg.   | 3(RBD) | VST   | 61    | 51    | 0.700      | 2222110000 3221100000  |
| J.L.                 | 2/13  | neg.   | 2      | SPT   | 58    | 44    | 0.730      | 2222100000 3441000000  |
| Carbon Sample 27-1.8 |       |        |        |       |       |       |            |                        |
| F.G.                 | 2/13  | pos.   | 2      | VST   | 59    | 98    | 0.727      | 5555532100 5532100000  |
| J.L.                 | 2/13  | neg.   | 2      | SPT   | 58    | 44    | 0.730      | 2222100000 0000000000  |
| J.V.                 | 2/13  | neg.   | 1      | ST    | 79    | 65    | 0.723      | 1111000000 2411000000  |
| Carbon Sample 15     |       |        |        |       |       |       |            |                        |
| L.G.                 | 11/14 | pos.   | 7(RBD) | T     | 62    | 74    | 0.588      | 5555353210 5554200000  |
| F.B.                 | 11/14 | neg.   | 4      | SPT   | 66    | 33    | 0.602      | 1111110000 5531000000  |
| R.D.                 | 11/14 | neg.   | 2      | VST   | 70    | 37    | 0.624      | 1111000000 5420000000  |
| A.C.                 | 11/14 | neg.   | 1(RBD) | SPT   | 77    | 34    | 0.620      | 0001110000 5531000000  |
| Carbon Sample 23-1.8 |       |        |        |       |       |       |            |                        |
| E.A.                 | 2/27  |        | 5      | VST   | 65    | 34    | 0.724      | 5555310000 5432100000  |
| C.M.                 | 2/27  |        | 1      | SPT   | 22    | 30    | 0.659      | 0000000000 4210000000  |

\*Tuberculous Meningitis.

added to each 100 ml. of carbon Sample 26. It is evident that the addition of carbonate changes the character of the colloidal suspensions, so that it is possible to regulate the sensitivity in such a way that the carbon solutions will give curves very similar to those obtained with the gold sols.

In Table VI we have recorded the complete data for 54 spinal fluids. It will be noted that the carbon curves parallel those of the gold sol. In the normal spinal fluids there is as a rule precipitation only in the first two tubes with the carbon solutions, whereas slight changes occur in many of the gold dilutions. In the fluids obtained from patients suffering from meningitis, the carbon curves offer still more striking contrast to those obtained with normal fluids inasmuch as the former show a protective action in the first tubes of the series.

The observations recorded in the foregoing tables are such as to indicate that colloidal carbon solutions can be prepared which may be used as test media for spinal fluids. The development of a standard technic to cover all possibilities demands further experimentation with tests of many spinal fluids and many different carbon samples, and will be the subject of a future paper.

#### SUMMARY

Colloidal carbon solutions suitable for use with spinal fluids can be prepared by electrolysis between two carbon electrodes in a 0.033 N Chromic acid bath.

The optimum sensitivity and stability is obtained at 30 volts and 3 amperes.

The sensitivity of the colloid can be altered by the addition of small amounts of 10 per cent sodium carbonate solution.

The curves obtained with colloidal carbon solutions are shown to be similar to those obtained with the gold sols.

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# A METHOD FOR THE SEPARATION OF THE PRINCIPAL CONSTITUENTS OF BILE<sup>2</sup>

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## INTRODUCTION

QUANTITATIVE studies of the various constituents of bile have been a matter of considerable difficulty. Bile is one of the most complex and variable of body fluids. Whipple<sup>1</sup> has pointed out the wide variety of chemical compounds which may be found. Wide ranges in the concentration of any constituent may occur, in biles from the same species, in liver and in gallbladder bile from the same animal, and in addition, there is a marked difference in composition from one species to another.

Three of the most important groups of compounds which occur in all types are the pigments, the bile salts, and the sterols. The bile pigments include bilirubin, biliverdin, and other pigment derivatives. The bile salts form one of the most complex groups of compounds which occur biologically. Most animal biles contain some or all of the conjugated bile salts, combinations of glycine or taurine with cholic or deoxycholic acids. In addition, other bile acids may occur which are more or less characteristic of the species. Human bile, for example, may also contain lithocholic and chenodeoxycholic acids (Ingold<sup>2</sup>). By virtue of their complex nature certain bile acids have a marked tendency to form more or less stable complexes; deoxycholic acid with fatty acids forming the choleic acids; complexes have also been postulated with lecithin and with cholesterol. The bile sterols include free and ester cholesterol and probably also oxysterol.

The physical and chemical properties of these constituents are such that even a trace of one compound may interfere with the accurate quantitative estimation of another. Two examples of such interference will illustrate the difficulties.

The estimation of cholesterol has presented special difficulties. Not only is it necessary to prevent the removal of any bile salts during the extraction of cholesterol from a sample of bile, but also no pigments must be in the final extract. The presence of a tinge of yellow, due to the extraction of a trace of some pigment derivative as reported by several workers (McLure and Mortimer,<sup>3</sup> McMaster<sup>4</sup>), will interfere markedly with the accuracy of the final colorimetric estimation.

The Pettenkofer reaction has commonly been used for the estimation of

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the bile acids although only acids of the cholic group may be thus estimated (Reinhold and Wilson<sup>5</sup>). Bile pigments again interfere with the production of the characteristic color and hence with the accuracy. Attempts have been made to overcome this defect by selective adsorption of the interfering substances (McLure, Vance and Greene,<sup>6</sup> Aldrich and Bledsoe<sup>7</sup>). The deficiencies of such methods have been pointed out by Gregory and Pascoe<sup>8</sup> who have advocated a monochromatic light to eliminate the interference in the final colorimetric estimation.

It is obvious that a greater accuracy could be expected in such colorimetric estimations if the desired constituents were separated completely from all other substances in the specimens of bile. Selective adsorption provides such a means of separation. Bile pigments, for example, have been adsorbed from urine on calcium carbonate, Hooper and Whipple,<sup>9</sup> and on barium sulphate (Cole,<sup>10</sup> Hunter<sup>11</sup>).

Few applications of such inorganic adsorptions to bile have been reported. McLure and others<sup>12</sup> adsorbed the bile pigments from duodenal contents on calcium sulphate. Wit<sup>13</sup> has recently studied the effectiveness of such compounds and found that ferric hydroxide and aluminum hydroxide completely clarified bile. Chiray and Cuny<sup>14</sup> have recommended neutral lead acetate for this purpose. Peoples,<sup>15</sup> more recently, has used calcium hydroxide to remove substances other than bile salts from bile prior to their estimation.

An extension of the principle of selective adsorption and preferential extraction should make it possible to separate several constituents from the same specimen of bile so that each might be determined. The method detailed below provides a simple method for the separation of the bile pigments, bile acids, and cholesterol from the same specimen of bile.

Briefly, the bile pigments are adsorbed on zinc hydroxide in aqueous solution. After many adsorbents had been tried this proved the most satisfactory since the reagent completely adsorbed the pigments at a slightly alkaline reaction. This is essential since the free bile acids precipitate out in an acid medium. Zinc hydroxide has the added advantage that it is a protein precipitant.

The greater part of the bile salts remain in the supernatant liquid. The fraction which is loosely adsorbed on the zinc hydroxide precipitate is removed by extractions, first with water, then with ethyl alcohol, while the pigments are not so removed. The latter solvent also extracts the cholesterol. In order to prove the completeness of extraction it was assumed that all bile acids were extracted similarly although, as Cuny<sup>16</sup> pointed out, the oxyacids give little or no color with the reagents for the Pettenkofer reaction. A negative test, especially using the more sensitive modification of an alcoholic solution of the bile salts with furfuraldehyde and sulphuric acid, was accepted as indicative of complete removal of the bile salts.

Repeated tests have confirmed the fact that the more common bile salts, at least, are insoluble in petroleum ether. The other common solvents such as chloroform, carbon tetrachloride and ethyl ether tended to extract traces of substances which produced a yellowish or brownish color with the reagents for



the Liebermann-Burchard reaction for cholesterol. This solvent was recommended by Elman and Taussig<sup>17</sup> for extracting cholesterol from whole bile. A negative Liebermann-Burchard test was accepted as indicating complete extraction of the cholesterol. The quantitative applications of this method of separation, especially with reference to the bile salt fraction, will be reported shortly.

#### METHOD

##### 1. Reagents.—

1. 0.5N potassium hydroxide.
2. Ten per cent zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ).

This is the concentration of alkali and zinc sulphate recommended by Somogyi<sup>18</sup> for the deproteinization of blood. Potassium hydroxide has been substituted for the sodium hydroxide since its carbonates and sulphates crystallize with little or no water of crystallization. The two solutions are related, however, as recommended, so that 10 c.c. of the zinc sulphate are neutralized to phenolphthalein by 10.8 or 11.2 c.c. of the alkali.

3. Ninety-five per cent and absolute ethyl alcohol.
4. Petroleum ether, boiling point 30-80° C.
5. Approximately 4N (22.4 per cent) potassium hydroxide.
6. Approximately 2N (14 per cent) potassium carbonate ( $\text{K}_2\text{CO}_3$ ).
7. Approximately 5N sulphuric acid, 1.39 c.c. concentrated sulphuric acid per liter.
8. Six per cent potassium hydrogen phosphate ( $\text{K}_2\text{HPO}_4$ ).

2. *Procedure*.—The quantity of bile required depends upon the constituent being determined. It has been found convenient to use such an aliquot of bile as will provide at least 25 mg. of bile salts or 0.5 mg. of cholesterol. This is usually achieved by using 1.0 c.c. or less of gallbladder bile or from 2.0 to 4.0 c.c. of fistula bile.

The quantity of bile must be carefully measured, preferably using a "to contain" pipette, into a 50 c.c. centrifuge tube. The pipette is rinsed two or three times with distilled water, using sufficient to bring the total volume to 10 c.c. Add 1 c.c. of the 0.5N potassium hydroxide, then add 1 c.c. of the zinc sulphate with gentle mixing. The contents of the tube are thoroughly mixed to obtain maximum adsorption of the pigment and precipitation of the protein. The tube is centrifuged for about five minutes. If the supernatant is not water clear, the addition of a few drops of the alkali followed by a similar amount of the zinc sulphate, will complete the adsorption. It is advisable to loosen any particles of the green precipitate from the upper portion of the tube by means of a fine stirring rod before the final centrifuging. The water-clear supernatant is decanted off into an Erlenmeyer flask.

To the precipitate is added about 9 c.c. of distilled water and the flocculent precipitate is stirred up thoroughly with a stirring rod which is rinsed off with about 1 or 2 c.c. of water. The tube is centrifuged and decanted off as before. This aqueous extract is added to the original supernatant. A second similar extraction is made with distilled water. These aqueous extracts remove not only a portion of the bile salts but also the excess zinc which should be removed by precipitation. Add 1 c.c. of 2N potassium carbonate to the combined aqueous extracts, mix and filter through a fat-free filter paper into a 125 c.c. Erlenmeyer flask. Wash the precipitate 2 or 3 times with small portions of distilled water to remove any bile salts.

The remaining portion of the bile salts and the cholesterol are removed by at least five similar extractions with 95 per cent ethyl alcohol. The first two are at room temperature while for the others the tube is placed in a water-bath at 60 or 70° C. for about five minutes with occasional shaking to prevent the precipitate from settling out. All of these alcoholic extracts are combined with the zinc-free filtrate forming the bile acid-cholesterol fraction.

The separation of the cholesterol from the bile salts presents little difficulty. Add 5 c.c. of 4N KOH to the bile salt-cholesterol extracts and evaporate to about 20 c.c. on the steam bath. This requires about thirty minutes which is sufficient to hydrolyze the cholesterol

esters but not the conjugated bile salts. At the end of this time 5N sulphuric acid is added to the first permanent turbidity due to the precipitation of free bile acids. The solution is then cleared by the gradual addition of 2N  $K_2CO_3$ , 2 c.c. in excess being added. Evaporate to dryness on the steam bath. The last traces of moisture may be removed by drying at 110° C. for not over thirty minutes. At least three extractions with 10 or 15 c.c. of petroleum ether, boiling up each time, and filtering from any solid particles if necessary are required. The petroleum ether extracts may be evaporated to dryness, taken up in chloroform and the cholesterol determined in the usual manner.

Bile salts may be separated from the potassium sulphate and carbonate by at least 5 extractions with absolute ethyl alcohol as above. The bile salts may be then estimated by some suitable procedure. A method to be published shortly has been developed to estimate the bile salts in this fraction.

The pigments may be readily removed from the precipitate. Add about 5 drops of 0.5N KOH and 2 c.c. of 6 per cent  $K_2HPO_4$  to the precipitate in the centrifuge tube. Stir thoroughly with a stirring rod in order to dissolve the pigment and to precipitate the zinc as the phosphate. Water or 95 per cent alcohol is then added slowly and with stirring to a volume of about 10 c.c. Place the tube in a hot water-bath for about five minutes with frequent stirring to favor solution. After centrifuging a clear green solution and a pale precipitate will be obtained. A second extraction with alcohol or water will remove the last trace of pigment.

#### SUMMARY

A simple method has been developed to separate the bile pigments, bile salts and cholesterol from the same specimen of bile. Each of these fractions may then be estimated without the interference of other constituents.

The author wishes to express his appreciation to Dr. J. B. Collip and Dr. D. L. Thomson of the Department of Biochemistry for their many helpful suggestions and criticism.

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## A MODIFICATION OF THE LANGE COLLOIDAL GOLD TEST\*

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SINCE the introduction of the colloidal gold test by Lange in 1912 many modifications have been reported. Nearly all of these changes have been in connection with the preparation of the reagent while the actual performance of the test has remained practically unchanged. The difficulties encountered in the preparation of suitable gold solutions accounts for the interest shown in this particular phase of the test. At best the preparation of the reagent is tedious, time consuming, and the solutions often fail to meet the necessary requirements of sensitivity.

We found that, by changing the method of diluting the spinal fluid, the test could be conducted with one-half the amount of reagent. This results in a saving of time and material as twice the number of tests can be performed with the same amount of reagent.

A series of parallel tests were conducted on each spinal fluid using the original method with 5 c.c. of reagent and the modification outlined below using 2.5 c.c. of reagent. A total of 12 fluids were tested, of which 5 gave typical Zone I (paretic) curves, 4 gave typical Zone II (syphilitic) curves, and 3 were negative. The reactions in the modified test were practically identical with those in the original test. A slight difference in the degree of reduction in a few of the corresponding tubes of each test was noted. This difference when present was at the beginning or end of the curves and not sufficient to alter the readings. Neither test proved to be more or less sensitive than the other.

To obtain additional proof that the amount of reagent used in the test could be varied, providing the fluid tested remained in the proportion, several lots of reagent were titrated for sensitivity by the method recommended by Ramsey and Eilmann.<sup>†</sup> In each case five separate titrations were conducted using 5, 4, 3, 2, and 1 c.c. of reagent with a corresponding reduction in the amounts of sodium chloride solution. The amount of reduction or sensitivity titer was exactly the same in all set-ups. This shows that the test can be conducted with any amount of reagent providing the test fluid is used in the same proportion as recommended in the original test. We feel, however, that the

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\*From the Laboratories of the Graduate Hospital, University of Pennsylvania.  
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<sup>†</sup>Ramsey, Thomas L., and Eilmann, H. J.: The Preparation of Colloidal Gold Solution, *J. LAB. & CLIN. MED.* **18**: 298, 1932.

amounts recommended below are more practical than smaller amounts, due chiefly to the possible introduction of error when dilutions are made with less than one-tenth cubic centimeter of spinal fluid.

Although the results obtained in these tests would be expected, as there was no logical reason to the contrary, yet one is reluctant to modify in the least a well-established technic of such an important test without first obtaining proof that such a change would not in some way interfere with its accuracy.

We have since used the modified method outlined below routinely with very satisfactory results.

*Modified Colloidal Gold Test.*—

1. Place eleven chemically clean test tubes in a rack.
2. Into the first tube place 1.8 c.c. of 0.4 per cent sodium chloride solution and 0.5 c.c. in each of the remaining 10 tubes.
3. Add 0.2 c.c. of spinal fluid to the first tube and thoroughly mix. (We feel that the dilutions will be more accurate if 0.2 c.c. of fluid is diluted with 1.8 c.c. of salt solution rather than 0.1 c.c. of fluid with 0.9 c.c. of salt solution. If the latter is used, the 1 c.c. is not discarded from the first as directed in Step 4.)
4. Discard 1 c.c. from the first tube, then transfer 0.5 c.c. to Tube 2; mix thoroughly and remove 0.5 c.c. and place in Tube 3; continue until the tenth tube is reached and then discard 0.5 c.c. from this tube. The eleventh tube is used as a control.
5. Add to each tube 2.5 c.c. of colloidal gold solution.
6. Mix thoroughly and set aside for twenty-four hours. The readings are now made and recorded the same as in the original test.

CONCLUSION

1. The Lange colloidal gold test can be conducted with varying amounts of reagent providing the proportion of spinal fluid remains the same.
2. A modification of the test is recommended which requires but one-half the amount of reagent that is used in the original method.

# A DROPPER FOR PERFORMANCE OF FRAGILITY TEST OF RED BLOOD CORPUSCLES\*

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IN THE performance of the red blood cell fragility test much difficulty was experienced in obtaining the desired dilutions of half percentage saline and distilled water when using the ordinary pipette for counting the number of drops.

To prepare the different strengths of saline solution by the drop method requires that the count of the number of drops of saline delivered to each test

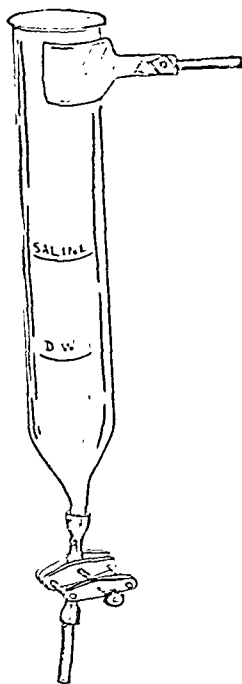


Fig. 1.

tube be absolutely accurate and that all drops be equal in size. The use of the apparatus to be described has decreased the time necessary for performance of the test, has simplified the mechanical manipulations, and has increased the dependability of the results.

A large test tube (1 inch in diameter and 6 inches long) is heated in the Bunsen flame and pulled until the cross-section at the narrowest part is about one-eighth of an inch. The glass is cut at the desired point so that a two-inch section of small rubber tubing like that used in blood-counting pipettes may be

\*From the Temple University Hospital.

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pulled over it and firmly held by its own elasticity. A short section of glass tubing (about 1 mm. inside diameter) is attached to the lower end of the rubber tubing and an adjustable screw clamp is placed on the middle of the rubber tubing, completing the apparatus.

When performing the test, the apparatus is fastened in a ringstand and filled with the solution to be measured. The clamp is loosened until a drop of fluid forms on the tip of the glass nozzle every eight or ten seconds. If now the lower end of the rubber tube and the glass tip be pulled downward, putting the rubber tube on the stretch, the rate at which the fluid runs through the tube will increase roughly proportional to the amount of tension on the hose, the solution falling regularly from the glass tip in drops of uniform size. Since the tension on the rubber tubing is so easily controlled with one hand and the flow is so uniform, the drops can be very easily counted and the flow instantly stopped at the desired moment. Meanwhile the other hand remains free to slide the test tube rack and its tubes consecutively under the dropper until each tube has received its predetermined number of drops of liquid. Saline and distilled water may be measured through the same apparatus with only a rinsing between.

A few trials will show the operator just how much saline and how much distilled water is necessary for the test, so that file marks may be placed at the proper levels on the large test tube as a guide in conserving these substances. When not in use the apparatus should be rinsed, the clamp loosened, and the whole laid away for future use.

3401 NORTH BROAD STREET

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## SEDIMENTATION TIME OF BLOOD\*

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### IMPROVED APPARATUS FOR ROUTINE OR RESEARCH TESTS

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THE expense of the graduated test tubes usually employed in determining the sedimentation rate of blood has been saved by the use of the apparatus described, which uses ungraduated test tubes which are read against a graduated background. The technic of the test remains essentially the same as with graduated tubes.

The apparatus consists of two rectangular pieces of wood or other substance so fastened together as to form a baseboard upon which the test tubes stand and a backboard which supports the tubes and which bears a millimeter scale easily drawn with the aid of a straight edge and millimeter rule. One horizontal line is drawn 51 mm. above the plane upon which the test tubes rest, i.e., the baseboard. This line, which is marked "0," corresponds to the level to which the blood citrate solution appears in the tube when properly filled. The other mark-

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\*From the Temple University Hospital.

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ings are made parallel to this "0" line at 10 mm. intervals. Finer lines may be drawn at 5 mm. intervals if desired. The completed scale (see Fig. 1) is used for determining the level between the cells and plasma in the tubes, which may be read directly to 5 mm. and to 1 mm. by interpolating between the nearest lines of the scale. Horizontal lines at millimeter intervals are easily ruled, but to us seemed unnecessary and confusing. The scale is most easily drawn on white paper with waterproof ink, carefully fastened to the backboard in the desired position and then covered with a piece of transparent celluloid film such as is used as a base in photography and radiography.

The test tubes are conveniently held in place with a continuous piece of soft brass wire which will not rust, become loose, or pull out. The lower row of wire loops should be placed about 7 mm. from the baseboard, instead of in the

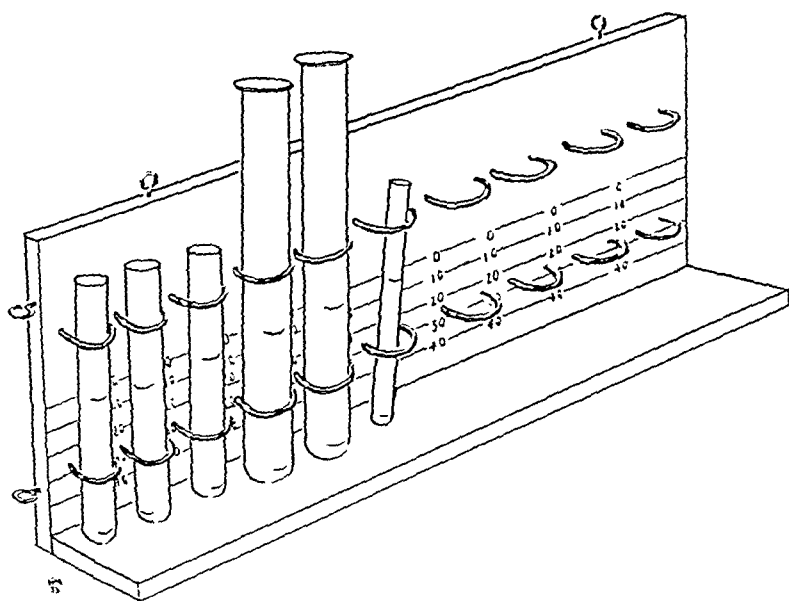


Fig. 1.

position shown in the illustration. When in the lower position they do not interfere with reading the scale, yet securely hold the tubes—the first requisite of any test tube rack. As shown, this rack accommodates tubes having an inside diameter of 15 mm., or any tube of smaller caliber. This feature enables one easily to study the effect of tube diameters upon the rate of fall without the necessity of graduating the tubes themselves. A set of eyes in the upper edge of the backboard will hold the rack at eye level when fastened to hooks or nails at the proper height on the wall. A set of eyes on the side is useful in swinging the rack from vertical fixtures such as pipes or the corner of a projecting shelf where the rack would be placed more conveniently than if it were against the wall. Since the tubes are against the scale against which they are read and the rack is at eye level, there is a negligible error in making readings in this way. While the rack was made of quarter inch pine board, I should think Bakelite would be the ideal substance.

In our sedimentation tests the 11 mm. tubes were used for routine, although larger and smaller tubes were used experimentally. Regardless of the size of the tubes, they may be prepared in the same way. With a millimeter rule, a single file mark is made 7 mm. from the base upon which the tubes rest and another one 51 mm. from the base. The file mark is easily filled with colored crayon or India ink and the excess wiped off, leaving a mark clearly visible.

In use, 2 per cent sodium citrate is dropped into the tube until the meniscus reaches the lower mark and blood is added until the meniscus of the mixture reaches the upper mark. The amount of citrate should be carefully measured because any appreciable variation from this standard amount will change the rate of fall to a greater extent than will any of the other technical procedures.

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### NOTE ON THE PROCEDURE IN THE KUTTNER-LICHTENSTEIN MICROCOLORIMETRIC METHOD FOR THE DETERMINATION OF ORGANIC PHOSPHORUS\*

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THE microcolorimetric method, developed by Kuttner and Lichtenstein<sup>1</sup> for the determination of organic phosphorus, involves digestion with sulphuric acid, aided by 30 per cent hydrogen peroxide of which the excess must be removed, and addition of sodium molybdate and stannous chloride to form a blue color. The only uncertain and tedious step in the procedure is the removal of excess hydrogen peroxide.

In the original method this was accomplished by adding water to the cleared digestion mixture, boiling down, adding more water, and repeating the process a number of times. From two to four hours are required to decompose the hydrogen peroxide completely, and almost constant attention is necessary. An alternative method, requiring little attention, consists of keeping the tubes in a hot water-bath for two or three days, and replacing the evaporated water from time to time.

The present modification eliminates both the tedious and time-consuming factors. It is carried out in a simple way as follows:

After the digestion mixture has been finally cleared (for some organic materials this may take a few additions of hydrogen peroxide with subsequent heating after each), it is digested for three minutes after the first white fumes appear. Care should be taken not to lose sulphuric acid by excessive fuming, since it has been shown<sup>2</sup> that, if the acid concentration in the final blue solution is less than 0.9 N, additional color is produced. Accordingly, the flame should be regulated so that white fumes appear in the bottom of the tube, but condense farther up.

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At the temperature of the boiling concentrated sulphuric acid, the three-minute period is sufficient to decompose the excess hydrogen peroxide. The excessive time required in the original procedure is the result of the relative stability of hydrogen peroxide at the boiling temperature of the dilute acid.

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## A SIMPLE TECHNIC FOR FINDING COCCIDIOIDES IMMITIS\*

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RECENTLY, while searching fresh moist preparations of sputum from a case of coccidioidal granuloma, for the characteristic spherical bodies, I found that they showed a strong iodine staining power. If enough Lugol's or Gram's iodine solution be mixed with the sputum or sinus tract pus to

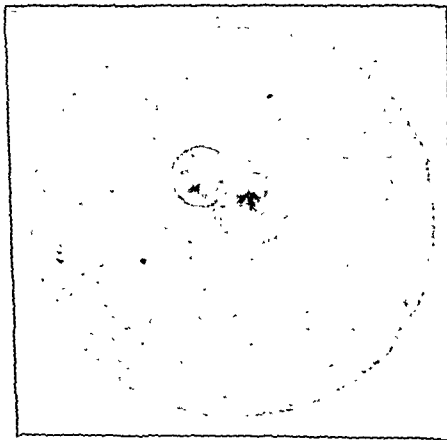


Fig. 1.—*Coccidioides immitis*, iodine stain ( $\times 640$ ).

color the other cells a lemon yellow color, the coccidioidal bodies will take a rich brown tint within five minutes and can be located easily by the low power objective.

Having seen no reference to this simple trick, I offer it to those who have occasion to look for these organisms. A large amount of material can be searched very quickly, and fat droplets or air bubbles that are confusing, at least to those unfamiliar with the organism, can be quickly excluded.

Fig. 1 shows the strong contrast produced.

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## THE ESTIMATION OF ETHYL ALCOHOL IN BRAIN\*

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### I. ALCOHOL IN EMBALMED TISSUES

THE identification and quantitative estimation of ethyl alcohol in the embalmed body has heretofore been difficult, if not impossible. Yet tissues from embalmed bodies are often sent to the chemist with the statement that alcoholism is the probable cause of death, and the chemist is expected to establish the point. We, therefore, planned experiments in the hope of developing a dependable quantitative method, based upon the method of Gettler and Tiber.<sup>1</sup>

Briefly, the Gettler and Tiber method consists of a steam distillation of a weighed portion of the brain (500 gm.); an aliquot portion of the steam distillate is then treated with potassium dichromate and sulphuric acid, redistilled, and an aliquot of the resulting acetic acid distillate titrated against twentieth normal sodium hydroxide. The percentage of ethanol is then calculated in terms of the sodium hydroxide titration, 1 c.c. of twentieth normal sodium hydroxide being equivalent to 2.3 mg. ethanol.

Throughout, the procedure of Gettler and Tiber has been followed, except where modification has been necessary to accommodate the amount of tissue used, and where the purpose of the experiment required. Preliminary to the investigation of tissue, standard mixtures of formaldehyde, methanol, and ethanol were used. The concentrations of ethanol in these mixtures were approximately those found in brain tissue, viz., from 0.4 per cent to 0.6 per cent. The method of removal of methanol and formaldehyde is based on the ease of oxidation of these compounds to carbon dioxide and water. Acetic acid, the oxidation product of ethanol, is extremely stable.

Direct distillation of the mixture from dichromate and sulphuric acid was first tried. On titration the results were quite variable, and in most instances, the values were much higher than was warranted by the amount of ethyl alcohol present. It was assumed that the increased acidity above that for acetic acid was due to formic acid, and no qualitative estimation of the oxidation products was undertaken. This experiment was repeated with the introduction of a preliminary period of refluxing before distillation. Upon distillation and titration the results were in agreement with the amounts of ethanol present in the original mixtures. This was accounted for by the fact that in refluxing, the methanol and formaldehyde are oxidized to carbon

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†Deceased.

dioxide and water, while the oxidation product of ethanol, namely acetic acid, is stable in the oxidizing mixture. The above results were duplicated many times.

The time of refluxing was varied. It was found that from thirty to forty-five minutes at a slow boiling point was sufficient for complete oxidation.

The above procedure was then applied to brain tissue of rabbits. The animals ranged from 1.5 to 3.3 kilograms in weight.

Five rabbits were used for an estimation of a possible normal alcohol content of the brain. They were housed for a period of one week prior to use, and fed upon carrots, lettuce, and whole bran.

The animals were killed by the neck stroke, and the brain removed, placed in an agate mortar, and thoroughly macerated. The macerated tissue was then washed into a 2 liter flask to which were added 300 c.c. of distilled water, a few drops of mineral oil and 1 c.c. of saturated tartaric acid solution. The flask was then connected to a steam still fitted with a Cummings bulb, and 300 c.c. of distillate was recovered. The entire steam distillate was placed into a 500 c.c. flask to which 20 gm. potassium dichromate and 40 c.c. of concentrated sulphuric acid were added. The flask was connected to an upright spiral condenser and 250 c.c. of distillate recovered. One hundred cubic centimeters of this distillate were titrated against N/100 NaOH. The titrations never exceeded 2.5 c.c. of the standard solution. An aqueous acid control distillation carried out in the same manner gave the same titration figures. Due to the agreement in blank and titration of brain distillates, it was assumed for the purpose of the experiment that the brains were negative for alcohol.

A second group of 5 rabbits were given by stomach tube, ethyl alcohol at a dosage of 6 gm. of ethanol per kilogram weight. The alcohol solution was of the strength of average alcoholic beverages (brandy, 50 per cent). The animals were killed, at one, two, five, ten, and twenty-four hours after the alcohol administration and the brains analyzed for alcohol, to determine roughly the time for development of maximum alcohol concentration in the brain. Table I shows the results of the experiment.

A second group of 13 rabbits were given the alcohol solution in the same dosage (6 mg. per kg.). Five animals were killed after an interval of five hours and the ethanol content of the brain estimated immediately; 8 animals were killed and embalmed immediately, and the brain removed for analysis one hour later. Two more animals received half the above dosage (3 mg. per kg.) and were killed five hours later for analysis of their brains. Finally,

TABLE I

| RABBIT | TIME ELAPSED | PER CENT ETHANOL |
|--------|--------------|------------------|
| 4      | 1 hour       | 0.321            |
| 10     | 2 hours      | 0.325            |
| 11     | 5 hours      | 0.359            |
| 10a    | 10 hours     | 0.278            |
| 12     | 24 hours     | 0.0031           |

three animals received this latter dosage, were killed after five hours and were embalmed one hour prior to removal of the brain. Table II shows the results of the experiment.

A control group of 5 animals were then killed in the same manner and embalmed. Upon analysis of the brain tissue the results were in accordance with those which were determined upon fresh brain tissue of control rabbits, the figures checking closely with blank titrations.

#### SUMMARY AND CONCLUSIONS

By modification of the Gettler and Tiber technic, introducing a period of refluxing of the dichromate-sulphuric acid step in the procedure, formaldehyde and methanol may be completely removed, so that only the ethyl alcohol fraction, namely acetic acid, appears in the final distillate for titration. Rabbits were used for the experiments. It seems safe to conclude that by this method, quantities of alcohol of medicolegal importance can be determined in brains of embalmed rabbits, and probably of embalmed human brains.

#### II. A MODIFICATION OF THE GETTLER AND TIBER METHOD FOR TRACES OF ALCOHOL

In the routine analysis of human brains for alcohol in the laboratory of the Los Angeles County Health Department, wherein the method of Gettler and Tiber was employed, more than 100 brains were tested whose titration figure was 0.9 c.c. or less of 0.05N NaOH. Such brains were reported to the coroner as negative for alcohol. Recalling, however, that Gettler and Tiber found an average of 0.0015 per cent alcohol in normal brains by correcting for organic acids in blanks similarly obtained, an effort was made to modify the method to reduce the total titration instead of correcting for it. The first

TABLE II

| UNEMBALMED BRAIN<br>6 GM. ALCOHOL PER KG. |                  | EMBALMED BRAIN<br>6 GM. ALCOHOL PER KG. |                  |
|-------------------------------------------|------------------|-----------------------------------------|------------------|
| NO.                                       | PER CENT ETHANOL | NO.                                     | PER CENT ETHANOL |
| 20                                        | 0.36             | A.                                      | 0.36             |
| 22                                        | 0.41             | B.                                      | 0.28             |
| 23                                        | 0.38             | D.                                      | 0.21             |
| 24                                        | 0.41             | E.                                      | 0.52             |
| 25                                        | 0.45             | F.                                      | 0.38             |
|                                           |                  | 26                                      | 0.32             |
|                                           |                  | 27                                      | 0.44             |
|                                           |                  | 28                                      | 0.24             |
| 3 GM. PER KG.                             |                  | 3 GM. PER KG.                           |                  |
| 29                                        | 0.15             | 31                                      | 0.16             |
| 30                                        | 0.15             | 32                                      | 0.14             |
|                                           |                  | 33                                      | 0.078            |

distillate from the brain contains alcohol and certain titrable organic compounds; the second distillate contains similar titrable substances in addition to the acetic acid, resulting from the oxidation of the alcohol. Gettler and Tiber calculated the normal alcohol present by subtracting the acidity of the first distillate from the acidity of the second distillate. The present study was based upon the supposition that the insertion of an alkaline distillation between

the first and second regular distillations would hold back the interfering materials. The procedure was as follows:

Five hundred grams of tissue was steam distilled to collect 800 c.c. After filtration, 300 c.c. was treated with 20 grams of potassium dichromate and 40 c.c. of sulphuric acid and distilled to collect 250 c.c. Fifty c.c. was titrated for the report to the coroner. When the titration figure for the 50 c.c. was 0.9 c.c. 0.05N NaOH, or less, the remainder of the steam distillate (500 c.c.) was treated with 50 c.c. of 3N NaOH or the equivalent amount of  $\text{Ca}(\text{OH})_2$  for the purpose of forming non-volatile soaps of contaminating organic acids. This mixture was steam distilled to collect 500 c.c., 300 c.c. was treated with dichromate and sulphuric acid and distilled to collect 250 c.c. and the entire distillate titrated. The figure for the reagent blank of 0.65 c.c. 0.05N NaOH was subtracted. One c.c. 0.05N NaOH is equivalent to 0.0013 per cent alcohol. This factor includes the correction of 90 per cent recovery in the acid distillation besides the corrections of dilution and conversion of acetic acid to alcohol.

Distillations from seven brains have been tested by the above procedure, with results as indicated in table III.

Before further work on the problem was undertaken, the paper by Gettler, Niederl, and Benedetti-Pichler<sup>2</sup> appeared, showing the average alcohol content of seven brains to be 0.0004 per cent, with a range of 0.00027 to 0.00055 per

TABLE III

| TREATED WITH NaOH |                       |                        |                  | TREATED WITH $\text{Ca}(\text{OH})_2$ |                       |                        |                  |
|-------------------|-----------------------|------------------------|------------------|---------------------------------------|-----------------------|------------------------|------------------|
| NO.               | FIRST ACID DISTILLATE | SECOND ACID DISTILLATE | ALCOHOL PER CENT | NO.                                   | FIRST ACID DISTILLATE | SECOND ACID DISTILLATE | ALCOHOL PER CENT |
|                   | 0.05 N NaOH C.C.*     | 0.05 N NaOH C.C.       |                  |                                       | 0.05 N NaOH C.C.*     | 0.05 N NaOH C.C.       |                  |
| Blanks            |                       | 0.65                   |                  | Blanks                                |                       | 0.65                   |                  |
| 2940              | 3.5                   | 1.6                    | 0.0013           | 3006                                  | 4.5                   | 1.0                    | 0.0005           |
| 2959              | 5.0                   | 1.3                    | 0.0009           | 3007                                  | 4.0                   | 1.0                    | 0.0005           |
| 2968              | 3.5                   | 1.4                    | 0.0010           | 3008                                  | 4.0                   | 0.7                    |                  |
|                   |                       |                        |                  | 3019                                  | 3.0                   | 0.7                    |                  |

\*Calculated for 250 c.c. of distillate.

cent. It will be seen that when  $\text{Ca}(\text{OH})_2$  was used to prevent volatilization of organic acids in the steam distillation process, the alcohol values for two brains by the titration method approached the values obtained by Gettler, Niederl and Benedetti-Pichler, using much more refined technic. The titration figures for two other brains by the  $\text{Ca}(\text{OH})_2$  method were so close to the blanks that calculations of percentage would have had no significance.

It is suggested that the figures for the  $\text{Ca}(\text{OH})_2$  distillation are lower than those for the NaOH distillation because of the lower volatility of the resulting calcium compounds.

Because present circumstances prevent further investigation of this type, and because of the closeness of the figures obtained by the modified distillation process with those by the more difficult procedure of Gettler and his

associates, the present results with the small number of brains are reported. It is believed that the method is useful for the determination of minute amounts of alcohol in human tissue.

The authors wish to acknowledge the helpful guidance and encouragement of Dr. R. V. Stone, Director of the Bureau of Laboratories, Los Angeles County Health Department.

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1. Gettler, Alexander O., and Tiber, Arthur: The Quantitative Determination of Ethyl Alcohol in Human Tissues, *Arch. Path.* 3: 75, 1927.
2. Gettler, Alexander O., Niederl, Joseph B., and Benedetti-Pichler, A. A.: The Isolation of Pure, Anhydrous Ethyl Alcohol from Non-Alcoholic Human and Animal Tissues, *J. Am. Chem. Soc.* 54: 1476, 1932.

## THE DIAZO REACTION FOR DETECTION OF CERTAIN LOCAL ANESTHETICS IN URINE AND IN TISSUES\*

W. E. GIBB, B.S., M.S., AND WILLIAM M. DEHN, PH.D., SEATTLE, WASH.

EGGLESTON and Hatcher<sup>1</sup> showed that procaine is rapidly destroyed by the liver. Thieulin<sup>2</sup> found that both it and its hydrolytic products were eliminated in the urine of animals. We have found that it can easily be detected in the urine a few minutes after injection in the tissues by application of Riegel's and Williams'<sup>3</sup> modification of the diazo reaction. In its most simple form one needs only to add in order a little sodium nitrite, acetic acid, and ammonia to observe the red or yellow formed, and compare the color with that of untreated urine. Any change of color is proof of the presence of procaine, etc., or their hydrolytic products such as meta or p-aminobenzoic acids, which because of contained amino groups also give colors with the reagent.

In a cadaver known to contain procaine, comminuted tissues made acid in reaction and then extracted with ether gave colors identical with the colors produced with the extracts from tissues made alkaline in reaction. This observation proves that procaine is hydrolyzed in the body, either before or after death. For this reason accurate quantitative analyses of tissues for procaine must involve the separation of procaine and its hydrolytic product, p-aminobenzoic acid.

In Table I are recorded data on the diazo reaction as applied to 1,233 specimens of urine examined in a hospital over a period of three months. All these patients had received medicine as administered in hospital practice, and only about 5 per cent of them had received procaine.

Seven of the patients showing apparent positive test for procaine, but not receiving procaine, were found to have gallbladder disease; these urines gave positive Van den Bergh tests for serum bilirubin. The other two patients did not return for further investigation. When apparent positive tests were indicated, the colors were not as distinct as in the tests following administration of procaine.

\*From Virginia Mason Hospital, and Chemistry Laboratory, University of Washington. Received for publication, June 21, 1933.

TABLE I  
NUMBER PATIENTS RECEIVING PROCAINE

|                                                              |    |  | POSITIVE AFTER |       |       | NEGATIVE |
|--------------------------------------------------------------|----|--|----------------|-------|-------|----------|
|                                                              |    |  | 1 HR.          | 2 HR. | 3 HR. |          |
| $\frac{1}{2}\%$ , 30 c.c.                                    | 17 |  | 14             | 2     | 1     | 0        |
| $\frac{3}{4}\%$ , 30 c.c.                                    | 2  |  | 1              | 0     | 0     | 1        |
| $1\frac{1}{2}\%$ , 6-12 c.c.                                 | 39 |  | 38             | 1     | 0     | 0        |
| $4\%$ , 12 c.c.                                              | 2  |  | 2              | 0     | 0     | 0        |
| $2\%$ , 2 c.c.                                               | 4  |  | 4              | 0     | 0     | 0        |
| TOTAL NUMBER EXAMINED                                        |    |  |                |       |       |          |
| Urine examined                                               |    |  |                |       |       | 1,233    |
| Receiving procaine                                           |    |  |                |       |       | 64       |
| Positive first hour                                          |    |  |                |       |       | 59       |
| Positive second hour                                         |    |  |                |       |       | 3        |
| Positive third hour                                          |    |  |                |       |       | 1        |
| Procaine negative (urine lost)                               |    |  |                |       |       | 1        |
| Not receiving procaine but giving apparent positive reaction |    |  |                |       |       | 9        |
| Negative urine                                               |    |  |                |       |       | 1,160    |

In all cases not showing a positive test during the first three hours there was evidence of impaired kidney function with some retention of urine. Three of these patients had a high blood urea and creatinine. Morning urine, which showed a high degree of color, showed no reaction or change in color in testing for procaine. As yet no specimens of urine except the seven mentioned above have given a test for procaine, except where procaine was given.

## SUMMARY

Riegel's and Williams' test is a quick and reliable test for procaine and other aminobenzoic acid derivatives used as local anesthetics, when excreted in the urine or when present in the cadaver. Over 1,100 specimens of urine of patients not receiving procaine gave negative tests, except that a few cases gave apparent test when gallbladder diseases were present. Hydrolytic products of procaine were found in the tissues of a cadaver.

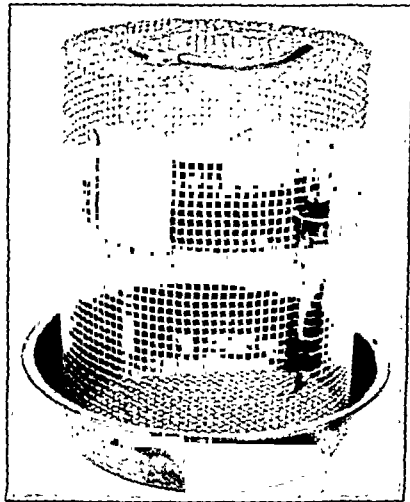
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## CAGE FOR MICE AND RATS\*

ALLAN W. BLAIR, AND EMMETT B. CARMICHAEL, UNIVERSITY, ALA.

THIS type of cage has been used by us for the past two years, and we feel that it possesses distinct advantages over other types of cages used heretofore. In our experience some of these advantages consist in the prevention of food and bedding pollution by the segregation of each in a different compartment, the ease and cheapness of construction, the greater accessibility of the animals, the ease of cleaning, the ease of observation of the undisturbed animal and the ease of transportation from place to place. The cage serves well for feeding experiments, breeding purposes, stock animals, and general experimental purposes. It is compact, economical of space, and may be



placed on a narrow shelf. Waste cotton is employed for bedding and it is placed in the upper compartment. If so desired, the food may be placed in the upper rather than the lower compartment.

*Dimensions.*—11.25 x 8.5 inches.

*Materials.*—Galvanized hardware cloth ( $\frac{1}{4}$  inch mesh) for body, door, removable floor, and as a guard around opening between the two compartments (to prevent bedding material from falling through into lower compartment); sheet zinc ( $\frac{1}{32}$  inch) for floor of upper compartment, card holder, and to form margin of door opening; sheet metal ( $\frac{1}{16}$  inch) for band at bottom of cage and door catch; heavy wire soldered to edge of mesh door (to provide additional strength and support); water container (6 inch test tube, single hole rubber stopper, glass tubing) fastened by detachable clips to side of body; enamel pan.

\*From the School of Medicine, University of Alabama.

Received for publication, August 2, 1933.

This article is being republished from the May issue because of the omission of the illustration.



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**DIPHTHERIA, Precipitated Toxoid as an Immunizing Agent Against,** Baker, J. N., and Gill, D. G. *Am. J. Pub. Health* 24: 22, 1934.

A single injection of 1 c.c. of precipitated toxoid has rendered 100 per cent of 197 Schick-positive children Schick-negative. Similarly, 1,400 of 1,414 children, or 99 per cent, were Schick-negative when tested on an average of from two to three months after a single injection. The original immunity status was unknown. Observations on 16,289 inoculations revealed 8 with abscess formation. As a rule local or general reactions were not severe.

**ERYSPELOID, Among Workers in a Bone Button Factory Due to the Bacillus of Swine** Erysipelas, Mc Ginnes, G. F., and Spindle, F. *Am. J. Pub. Health* 24: 32, 1934.

Two hundred and ten cases of an erysipeloid condition occurred in a bone button factory among individuals handling cattle or hog bones. There was a close correlation with the handling of wet bone and occupations in which injuries were frequent. Repeated attacks of the disease were frequent, indicating that there was little or no local or general immunity produced by an attack. The bacillus swine erysipelas was isolated from one case and from samples of bone, bone material, and bone waste collected at various stages in the manufacture of buttons. For ridding the plant of infection the heating of all bones entering the plant in the soaking vats to a temperature of 144° F. for at least two hours is recommended.

**TUBERCULOSIS, Blood Culture in,** Penfold, W. J., and Butler, H. M. *Med. J. Australia* 2: 837, 1933.

In three out of twenty cases of tuberculosis it was possible to cultivate *Bacillus tuberculosis* from the blood by Lowenstein's method. In the cultures from one other case acid-fast organisms could be identified microscopically by means of smears made from the surface of the medium; but these cultures, though incubated for six months, failed to produce colonies visible to the naked eye. Subcultures from them likewise failed to grow.

The Lowenstein method of blood culture in tuberculosis is troublesome and time-consuming in execution and gives so few positive results that the authors cannot believe that it is destined to render any great service to the clinician in the diagnosis of tuberculosis.

**DIABETES, Insulin Resistance in,** Mac Bryde, C. M. *Arch. Int. Med.* 52: 932, 1932.

Attention is called to the frequency of occurrence of relative resistance to insulin in uncomplicated cases of diabetes mellitus.

Clinical studies of two cases of insulin-sensitive and three cases of insulin-resistant diabetes are reported.

In two of these patients resistance to endogenous insulin was apparently broken, an overdosage of insulin being followed by marked improvement in carbohydrate tolerance.

The clinical characteristics of the insulin-resistant type as distinguished from the insulin-sensitive type are outlined.

Reported cases of insulin resistance may be grouped as follows:

(a) Unexplained, such as the five cases reported in this paper. Insulin resistance is

usually of mild or moderate degree in this group. Hyperactivity of the suprarenal-sympathetic system is suggested as the cause of this type of resistance. Many of these patients might be termed constitutionally resistant.

(b) Infection.

(c) Destructive pancreatic disease.

(d) Disorders of other endocrine glands, particularly the thyroid, pituitary and suprarenal glands.

(e) Hepatic disorders.

(f) Miscellaneous conditions such as acidosis, coma, diseases of the skin and cardiac decompensation.

The value of determining the relative responsiveness to insulin for treatment as well as for further study of diabetes is emphasized.

**MYELOSIS, Aleukemic, Baldridge, C. W., and Fowler, W. M.** Arch. Int. Med. 52: 852, 1933.

Ten cases are reported in which a common feature was myeloid hyperplasia.

Two cases were ordinary instances of leucemic myelosis, except that in the course of each there developed an almost completely aleukemic period. In one case the aleukemic period was thought to be due to irradiation of the spleen; in the other, it was "spontaneous."

Four cases thought to be diffuse hyperplasia of the myeloid tissue were characterized by severe anemia, leucopenia, a few abnormal leucocytes, secondary hemorrhagic purpura, changes in the bones and extramedullary collections of myeloid cells.

Two cases were indistinguishable from ordinary multiple myeloma, except that the tumors were made up of myeloid cells instead of plasma cells. One case presented chemical changes sometimes seen in multiple myeloma of the plasma cell type.

In the last two cases extramedullary myeloid tumors were present without definite evidence of disease of the bone marrow.

**ANEMIA, Macrocytic in Disease of Liver, Van Duyen, J. 2nd.** Arch. Int. Med. 52: 839, 1933.

An introductory case illustrating the occurrence of macrocytic anemia in cirrhosis of the liver is briefly reported.

References from the literature show this to be a widely recognized syndrome.

Three cases of disease of the liver are reported in which personal study has confirmed the macrocytic character of the anemia.

Twenty-eight cases of hepatic cirrhosis were reviewed from the records of the Syracuse University Hospital. Macrocytic anemia was found in 5 cases, or 18 per cent.

The mechanism producing a macrocytic type of anemia is discussed. Diseases of the liver is given a place in this mechanism.

**SCHULTZ-CHARLTON TEST, Fischer, A. E., and Kojis, F. G.** Am. J. Dis. Child. 46: 1282, 1933.

From a study of this reaction as well as the calcium blanching test in 486 cases the authors conclude that:

The Schultz-Charlton test is specific for the rash of scarlet fever.

A 10 per cent solution of calcium gluconate will cause slight blanching in some early cases of scarlet fever in which a mild or moderately intense rash is present. This action, however, is not so constant or so persistent as that obtained with dilute scarlet fever antitoxin.

The actions of calcium and of antitoxin on the rash of scarlet fever are totally different.

The fact that calcium will blanch a rash does not in any way detract from the validity of the Schultz-Charlton test.

The Schultz-Charlton test, provided its limitations are understood, is invaluable in the early diagnosis of scarlet fever.

**MALARIA, A New Antigen for Henry's Reaction, Livierato, S., Vagliano, M., and Constantacato, G.** *Greece Med.* 31: 177, 1933.

The sac of a cuttlefish with its contents is cut up into small pieces and put into a sterile tube; 40 c.c. of sterile distilled water are added, and 0.02 c.c. of formalin. After twenty-four hours at room temperature, the supernatant fluid is pipetted off; it is kept in the ice chest for two months before use, and is shaken daily. It is diluted to make a slightly blackish fluid just before use. The results are the same as those obtained with Henry's ox-eye melanin.

**SYPHILIS, Blood Cytology in Treated and Untreated, Rosahn, P. D., and Pearce, L.** *Am. J. M. Sc.* 187: 88, 1934.

From a study of the blood cytology of 126 treated and 87 untreated syphilis patients it was found that:

Treatment causes a fall in the total white cell count, the platelet count, and the relative and absolute numbers of neutrophils and monocytes, and a rise in the relative and absolute numbers of lymphocytes, and per cent hemoglobin, from values observed in the untreated cases.

No significant differences were noted between the mean blood cell values of the primary and secondary untreated patients.

A marked anemia was the outstanding feature of the blood cytology in the untreated tertiary disease. This anemia was absent in patients whose treatment was begun in the tertiary stage.

No differences in the blood cytology of treated patients were noted when treatment had been begun in the primary, secondary or tertiary stages of the disease.

A monocyte-lymphocyte ratio higher than 0.55 was more frequent among untreated patients and a monocyte-lymphocyte ratio less than 0.55 was more frequent among treated patients than would be expected from random association.

In a group of persistently treated patients, repeatedly negative serology was more frequently associated with a monocyte-lymphocyte ratio less than 0.45, with lymphocytes higher than 1,350 per c.c., and with monocytes less than 700 per c.c.; and persistently positive serology was more frequently associated with a monocyte-lymphocyte ratio higher than 0.45, with lymphocytes lower than 1,350 per c.c., and with monocytes higher than 700 per c.c. than would be expected from random association.

The changes observed in the cytology of the treated patients as compared with the untreated patients were similar in direction and affected the same cells as the changes observed in the spontaneously regressed experimental disease as compared with the period of lesion activity. This similarity lends additional weight to deductions drawn from the experimental disease as applied to human syphilis.

**ANEMIA, Aplastic, Analysis of So-Called, Thompson, W. P., Richter, M. N. and Edsall, K. S.** *Am. J. M. Sc.* 187: 77, 1934.

Thirteen cases of "aplastic" anemia have been studied. In all, the disease was characterized clinically by a progressive decrease in the number of erythrocytes, leucocytes and platelets, a progressive hypocythemia. In all, evidences of attempted regeneration were noted in the peripheral blood.

The marrow revealed a wide variation in microscopic appearance. In only 1 of the 13 was the marrow aplastic. In 2 it appeared hypoplastic, in 2 it was morphologically normal. In the remaining 8 there was moderate to marked hyperplasia of one or more of the formative elements.

It is felt that many of the restrictions and limitations placed on the clinical and pathologic diagnosis of "aplastic" anemia should be removed and that progressive hypocythemia alone should be sufficient reason for making the diagnosis, provided leucemia can be excluded.

The lack of correlation between the blood picture and bone marrow morphology has been discussed. It is felt that examination of the peripheral blood may be, at times, no index as to the condition of the blood-forming apparatus. Specifically, progressive hypocythemia as observed in these cases is not, in itself, sufficient evidence on which to postulate the presence of aplastic bone marrow.

This strongly suggests that it is quite possible to have serious interference with the normal processes of development, maturation and delivery of blood cells without evident alteration in the cellular content of the marrow.

**B. COLI HEMOLYTICUS, Clinical Significance of, Niles, W. L., and Torrey, J. C. Am. J. M. Sc. 187: 30, 1934.**

Strains of *B. coli* possessing hemolytic properties are frequently isolated from human feces (31 to 45 per cent of stools). They are somewhat more often found and in larger numbers in feces from patients with disorders relating to the digestive tract.

There is no distinctive difference either as regards morphology or cultural characteristics in the hemolytic *B. coli* strains isolated from the stools of normal or sick people, although those from the former group have seldom exhibited any virulence.

The authors have been successful in relieving the symptoms of toxemia by autogenous vaccines. *B. coli hemolyticus* has thus been eliminated from the feces in 93 per cent of their cases. They have recurred in 3 per cent after one year or longer.

Vaccination is the only method for eliminating *B. coli hemolyticus* from the human feces which the authors have found effective.

**TISSUE, The pH of Formalin—A Factor in Fixation, Burke, F. V. Am. J. Path. 9: 915, 1934.**

The following formula is recommended as superior and giving excellent results with all staining methods:

|                     |         |
|---------------------|---------|
| Distilled water     | 75 c.c. |
| Commercial formalin | 25 c.c. |
| Pure pyridine       | 5 c.c.  |

**BRUCELLA INFECTION, A Study of the Opsono-Cytophagic Power of the Blood and Allergic Skin Reaction in, Huddleson, I. F., Johnson, H. W., and Hamann, E. E. Am. J. Pub. Health 23: 917, 1933.**

The studies conducted on citrated blood of human beings who were known to have had undulant fever in past years and shortly after recovery, who are actively infected, or who have no history of the disease, show that the in vitro activity of the polymorphonuclear cells in whole citrate blood for Brucella is an expression of immunity to Brucella and an indication of the progress toward recovery in active infection. The absence of or a low phagocytic activity obtained in conjunction with a negative allergic skin test is evidence of susceptibility to Brucella infection. Infection in an individual is indicated by a positive allergic skin test obtained with Brucella nucleo-protein in conjunction with negative or low opsono-cytophagic activity of the whole citrated blood for Brucella.

The technic followed by the authors is described below:

**Determination of Opsono-Cytophagic Power of Blood:** The method adopted for determining the opsono-cytophagic power of blood for Brucella is a modification of the Leishman-Veitch technic and consists of mixing equal quantities of a citrated (0.8 per cent) fresh blood and a heavy bacterial suspension of living organisms in small Wassermann tubes, incubating at 37° C. for thirty minutes, and subsequently making spreads and staining with

Hastings stain. The addition of a definite amount of sodium citrate prevents clotting of the blood and inhibits the action of Brucella opsonins which are present in the serum of many normal individuals.

*Preparation of Blood Specimen:* The blood specimens are collected in 5 c.c. amounts in glass vials in which has been placed 0.2 c.c. of a 20 per cent solution of sodium citrate in physiologic salt solution. The final dilution of sodium citrate in the blood is 0.8 per cent. The test should be conducted on the specimens within six hours after collection provided they are kept in a cool place. The polymorphonuclear cells in blood disintegrate very rapidly when it is kept warm for two or three hours. The specimens should be thoroughly shaken directly before mixing with the bacterial suspension.

*Bacterial Suspension:* The bacterial suspension is prepared fresh each day by suspending several loopfuls of the growth from a forty-eight-hour liver agar slant culture in sterile physiologic salt solution of P<sub>H</sub> 7. The turbidity of the suspension should give a reading of approximately 2 cm. when measured with the Gates apparatus. Suspensions of any of the three species of Brucella are suitable regardless of virulence.

*The Test:* Into clean small glass vials, such as are used for the agglutination or Kahn test, are placed 0.1 c.c. of the whole blood and 0.1 c.c. of the bacterial suspension. After mixing thoroughly, the vials are placed in an incubator for thirty minutes at 37° C. Certain strains of Brucella tend to become "fast" to ingestion by the cells after being transferred daily for many weeks. If a culture is being used daily for the test, it should be checked frequently for "fastness" to phagocytosis against whole citrated blood from a known immune person. Continuous agitation during the period of incubation tends to inhibit phagocytosis. Considerable sedimentation of the blood cells takes place during the incubation period. The cells should not be resuspended by shaking after the period of incubation. Directly after removing the tubes from the incubator, a small amount of sedimented cells is removed by means of a finely drawn capillary pipette to which is attached a small rubber bulb. A drop of the cells is placed at one end of a thoroughly cleaned and polished glass slide, and drawn across the slide by placing the end edge of another slide at such an angle that the spread thins out and terminates at or near the middle. In a spread of this type, most of the leucocytes may be found near the terminating edge of the spread. The blood film should be dried as rapidly as possible to prevent shrinking of the leucocytes. Rapid drying may be obtained by placing the slides in front of a small electric fan. A small heating unit from an electric heater, if attached to the front of the fan and operated simultaneously, will greatly increase the speed of drying.

*Staining Spreads:* The slides are placed face upward on a suitable rack and the spreads covered with 0.5 c.c. of Hastings stain. After an exposure of fifteen seconds, 1 c.c. of distilled water is added to the stain on the slide. At the end of ten minutes, the spread is gently washed from stain with distilled water and dried in front of an electric fan.

*Estimation of the Degree of Opsono-Cytophagic Activity:* The size of the organism in question and the marked degree of phagocytosis which occurs in cells from immunes has necessitated the employment of a different system of recording phagocytic activity from that which is commonly used in studies of this nature. In routine work, a total of 25 cells is counted in different sections of the spread and each cell is recorded as negative when no phagocytosis occurs, as slight when from 1 to 20 bacteria are seen in the cell, as moderate when from 21 to 40 bacteria are found in a cell, and marked when the number of bacteria in the cell is above 40. The bacteria are so numerous in those cells showing marked phagocytosis that it is impossible to count all of them.

It is realized that the foregoing method of measuring degrees of phagocytosis is only approximate. It was adopted after making thousands of examinations by different methods.

## REVIEWS.

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### Influenza, With Special Reference to the Part Played by Pfeiffer's Bacillus, Streptococci, Pneumococci, Etc., and the Virus Theory\*

**I**NFLUENZA, because of its terrific morbidity and mortality, is, without doubt, one of the most important of all epidemic diseases. It is natural, therefore, that the literature concerning it has reached literally enormous proportions with little evidence of slackening in the flood of contributions.

While there has accumulated as a result a tremendous amount of information, it is questionable if it has ever before been gathered and correlated in one place.

To organize, abstract, and summarize this information is the purpose of this study, the completion of which will require two volumes.

In the present ninth volume of the *Annals of the Pickett-Thomson Research Laboratories*, which constitutes Part I of the Sixteenth Monograph from this source, the nomenclature, and history, as well as the clinical character of influenza, its varieties, symptoms, diagnosis, prognosis, mortality, and epidemiology are considered at length and in some detail.

In Part II, to be issued, the study will be rounded out by a consideration of the bacteriology of the complications of influenza as well as of the pathology, treatment, and prevention of the disease.

When completed, the monograph will represent a comprehensive survey of the literature of the investigations of this disease, summarizing the information contained in over 4,000 papers and present as well an account of the author's researches during the past fifteen years.

Those familiar with the previous monographs from this source will undoubtedly receive the present one with equal interest and recognize it as a contribution of undoubted value.

The rôle of Pfeiffer's bacillus is considered at length, the authors presenting as their own conclusion the belief that, while not the primary cause of true influenza, this organism plays an exceedingly important rôle in the production of the dangerous complications so commonly encountered in this disease.

They emphasize the importance and necessity for symbiotic studies in future investigations, an avenue hitherto subjected to but little consideration.

Like many others, the authors are strongly impressed by the symbiotic importance of streptococci in influenza and the fact that *B. influenzae*, streptococci and pneumococci when growing together enhance each other's virulence. They believe that the pneumococcus, while without special significance in the direct etiology of influenza, plays an important secondary rôle and directly influences the course of the infection. The evidence concerning the *Bacterium pneumosintes* they consider as yet inconclusive.

While the studies concerning the rôle of a filter-passing virus in the production of influenza are as yet incomplete and sometimes at variance, the Thomsons believe that the results of more recent work are consistent with the view that epidemic influenza in man is caused primarily by a virus infection, although further intensive study is needed.

Although space precludes any survey in extenso of this publication, enough has been said to indicate the importance of this contribution to what has been and, to a great extent is, one of the most baffling problems of medicine.

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\*Influenza, With Special Reference to the Part Played by Pfeiffer's Bacillus, Streptococci, Pneumococci, Etc., and the Virus Theory. By D. and R. Thomson, Pickett-Thomson Research Laboratory, London. Paper, pp. 640, 28 plates. The Williams & Wilkins Co., Baltimore, Md.

## Die physikalische Seite des Blutgerinnungs problems und ihre praktische Bedeutung\*

MANY are the theories that have been set up to explain the clotting of blood, many are the studies that have been made to determine the chemical processes involved. Practically, it is well known that the physical surface with which the blood is in contact has much to do with the clotting. In the live normal blood vessels the blood does not clot, in contact with the tissues and most foreign substances it does clot. However, the speed and the completeness of the clotting process vary greatly according to the surface with which the blood is in contact. Paraffin greatly delays clotting, glass produces rapid and strong clotting. A smooth, polished surface lessens clotting, a rough surface favors clotting. A substance with a low surface tension decreases clotting, high surface tension favors clotting. How can these facts be used practically? In the production of antitoxin a strong clot is desired to favor the full separation of serum; in the clinical transfusion of blood clotting is not desired. To lessen clotting, the needle with which the blood is drawn should be polished on the inside quite as well as on the outside as a rough surface favors clotting. The blood should not be allowed to touch glass as glass favors clotting by its high surface tension. A layer of paraffin on the surface of the glass lessens clotting but also lessens visibility. Lampert has found that amber lessens clotting almost as well as paraffin and is transparent. He hopes to find some substance that can replace glass entirely and practically eliminate the danger of clotting. Amber is a natural resin and now with all our synthetic resins such a hope may be realized.

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## Ergebnisse der medizinischen Strahlenforschung†

THIS stately sixth volume of 650 pages maintains the high standard set by the preceding volumes. Its 470 cuts are well selected and excellently printed on good paper. A wide range of subjects is covered as is shown by a few of the chapter headings: "Skin erythema and roentgen rays" by Alfred Reisner of Frankfurt. This is a study of the various factors in the production of erythema, such as the quality and quantity of the rays, the effect of divided doses, season of the year, etc. It has 44 cuts and about 220 references. "The roentgenotherapy of arthritis deformans," by Günther von Pannewitz of Freiburg. This is a study based on the treatment of more than 1,500 arthritic joints and experimentally induced arthritis in animals. It has 60 cuts and about 100 references. "Roentgen diagnosis in obstetrics" by Paul H. Schumacher of Giessen. This demonstrates how helpful the x-rays can be in obstetrics. It has 65 cuts and about 500 references. "The roentgenological demonstration of diverticula in the intestinal tract" by Ludwig Bayer and Hans Pansdorf. A rather complete monograph giving the anatomy, physiology, origin, clinical significance, and treatment. It has 77 cuts and about 300 references.

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## Handbuch der biologischen Arbeitsmethoden‡

THIS part 412 of this mammoth work contains chapters on determination of the respiratory quotient in surviving tissues, the various forms of the Helm respiratory apparatus, measurement of the basal metabolism with the Simonson apparatus, and an apparatus for the analysis of gases from respiration chambers for men and animals.

\*Die physikalische Seite des Blutgerinnungsproblems und ihre praktische Bedeutung. (The Physical Aspects of the Clotting of Blood and Their Practical Significance.) By Heinrich Lampert. Georg Thieme, Leipzig, 1931.

†Ergebnisse der medizinischen Strahlenforschung. (The Results of the Medical Investigations of Radiation.) Band 6. Georg Thieme, Leipzig, 1933.

‡Handbuch der biologischen Arbeitsmethoden, Lieferung 412. By Emil Abderhalden. Urban und Schwarzenberg, Berlin, 1933.

### Stedman's Medical Dictionary\*

HOW can one review a dictionary other than by remarking on the number of new words that have been added and concluding with a sophistry on the rapid growth of the language of the medical sciences? This can be done briefly. Over a thousand words have been added since the last edition three years ago. The language is growing by at least one new word a day. But in this book one does find more, deserving of mention. It is to be found particularly in the preface to the first edition, written twenty-five years ago. In it, the author backed by his wealth of previous editorial experience classifies himself unequivocally as a purist in etymology and nomenclature. He states his reason for his general principles of selection of spelling, clearly and logically. In the earlier days of this particular dictionary this was an uphill fight, in which the editors of some of the leading medical journals offered no cooperation. However, as the author states, the general principles for which he has contended have gradually been adopted not only in the general medical literature but also in the other two outstanding medical dictionaries.

The volume is an authoritative reference dictionary, which possesses a distinct advantage in that it is still being edited by the man who started it and who has still been able to develop it along the same clear-cut lines on which it was started and without changes of policy which sometimes tend to confuse the general plan.

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### Handbuch der Chemotherapie†

THIS is the first part of the great work on chemotherapy which proposes to gather up and present systematically all the work that has been done so far in this great field. This first part includes only the nonmetallic organic compounds, but it runs to 650 pages. It includes such important compounds as quinine, emetine, cod liver oil, germanin, chenopodium, salicylic acid, santonine, chaulmoogra oil, and hundreds of others. For all of the important ones it gives the chemistry, the preparations, the chemical derivatives, the pharmacology, the animal and clinical experiments, the uses and extensive references to the literature. For example, after 25 pages given to other chinoline derivatives, 84 pages are given to quinine and its derivatives with ten pages of references. Emetine gets 33 pages, chaulmoogra oil and similar unsaturated oils 35 pages, etc. It is a veritable mine of information.

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\*Stedman's Medical Dictionary. A Practical Medical Dictionary. By Thomas Lathrop Stedman, A.M., M.D. Editor of the "Twentieth Century Practice of Medicine" and of the "Reference Handbook of the Medical Sciences." Formerly editor of the "Medical Record." Twelfth edition, revised, illustrated, cloth, William Wood and Company, New York.

†Handbuch der Chemotherapie. Erster Teil. By Viktor Fischl and Hans Schlossberger. Fischers Medizinische Buchhandlung, Leipzig, 1932.



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## EDITORIAL

### The Laboratory Diagnosis of Undulant Fever

THE disease first known as Mediterranean fever and now recognized as indistinguishable in its etiology from "Bang's abortion disease" in cattle, has well borne out the prophecy of Nicolle who said of it: "Mediterranean (undulant) fever is in the course of evolution and is tending to become chronic. It is a malady which, on account of its manifestation and chronicity, will become one of the most common and stubbornest diseases. . . . Mediterranean fever is a disease of the future."

The correctness of this forecast is reflected in the now relatively voluminous literature of Brucellosis, as the infections due to this group of organisms, the *Brucella*, is now generally termed.

Recent interest and the modern investigations of undulant fever began with the appreciation of fact, first suggested by the work of Evans<sup>1</sup> and the

later studies of Meyer and Shaw,<sup>2</sup> and corroborated by those of other workers, that the organisms in this group, whether of human, porcine, caprine, or equine variety are all alike pathogenic for man and widespread in their distribution.

Whether they are each separate species or simply varieties of a single species is still under discussion and, for the clinician, possesses practically only an academic importance.

The fact that all are pathogenic for man and responsible for clinical syndromes by no means always clear cut or readily recognized is a matter of definite clinical importance; but the problem thus presented to the practitioner is fortunately rendered relatively easy of solution through the proper utilization of various laboratory procedures which have been developed and which are now more or less generally available.

Brucellosis, or undulant fever, in man may be seen in four types: malignant, intermittent, undulatory, and ambulatory not always, however, clear-cut or readily distinguishable as the disease has a marked tendency to change its type as it progresses.

The ambulatory and undulatory forms, together with the chronic and sub-clinical infections, are those most often presenting diagnostic difficulties, as the disease has not infrequently been found to be the explanation of obscure periodical fevers, arthritis, and intestinal and abdominal disorders and has been confused with tuberculosis and typhoid fever.

Very early in the study of human brucellosis the value and diagnostic significance of agglutination reactions became apparent so that this method was for some time regarded as having a value equal to, if not even greater than, that of the Widal test in typhoid fever.

Many studies soon showed, however, that the clinical interpretation and significance of positive agglutination reactions with *Brucella* antigen suspensions were influenced by a variety of factors.

Probably no one has been more closely associated with the investigation of brucellosis, nor has become more familiar with all its phases than Huddleson, whose studies, indeed, are responsible for much of the information now available concerning this disease and who may well be regarded as one of the outstanding authorities on the subject, especially as concerns its laboratory study and diagnosis.

Huddleson's rapid slide method for the performance of the agglutination test, for example, has long since been recognized as a standard procedure and his recent monograph<sup>3</sup> presents in an admirable and thorough manner the present status of the laboratory diagnosis of this disease.

The procedures of value in the laboratory diagnosis of undulant fever are: the agglutination test, the allergic test, and the estimation of the degree of opsono-cytophagic activity, all of which are especially valuable when, as in the majority of instances, the detection of the organisms by culture has not been possible or feasible.

The determination of agglutinins, whether in the serum in the case of man, or in the serum or milk in the case of animals is a procedure of great utility

which, by means of Huddleson's rapid slide method may be carried out practically anywhere.

When the macroscopic test tube method is used it must be recognized that the result may be influenced by a variety of factors among which, as determined by the work of Fitch and his coworkers<sup>4, 5</sup> and of Henry and Traum,<sup>6</sup> the more important are:

1. Hemoglobin in the serum, causing nonspecific agglutination.
2. Heating serum to 56° C. or over, which lowers its agglutination titer, an effect also produced by storage of the serum.
3. Excessive antigen turbidity which tends to decrease the titer of the serum.
4. Formalin or tricresol as preservatives which retard and may even inhibit agglutination.
5. Insufficient incubation of the test.
6. Presence of agar in the antigen, which may cause nonspecific agglutination.
7. Presence of thermolabile strains in the antigen which may be agglutinated by negative serum and not by positive serum.

While the rapid method of Huddleson, devised particularly for the examination of cattle, may also be used for tests of human serum, it is important to emphasize that the results cannot be given a similar interpretation as a negative reaction, even when repeated, does not necessarily signify an absence of infection, nor does a positive reaction even in high dilution furnish proof of an active infection, as it may be the result of past infection, either clinical or subclinical, or the result of exposure to infective materials, as in the case of veterinarians, dairy and packing-house workers and the like.

The results of agglutination tests in man must always be considered in the light of the history and other confirmatory tests.

The demonstration of allergic reactions as a method of diagnosis in the human being has been studied by various workers, Burnet<sup>7</sup> using a culture filtrate and Giordano<sup>8</sup> a killed suspension.

The determination of the opsonocytophagic power of the blood as an index of brucellosis was introduced by Huddleson, Johnson and Hamann<sup>9</sup> and bids fair to be definitely useful.

From the correlation of all these methods very valuable and clear-cut information may be obtained which has been presented by Huddleson<sup>10</sup> in the tabular form (Table I).

TABLE I

| AGGLUTINATION TEST | ALLERGIC SKIN TEST | OPSONO-CYTOPHAGIC POWER   | STATUS TOWARD BRUCELLA |
|--------------------|--------------------|---------------------------|------------------------|
| 0                  | 0                  | 0 - 20% of cells slight   | Susceptible            |
| 0                  | +                  | 0 - 40% of cells marked   | Infected               |
| +                  | +                  | 0 - 40% of cells marked   | Infected               |
| 0                  | +                  | 60 - 100% of cells marked | Immune                 |
| +                  | +                  | 60 - 100% of cells marked | Immune                 |

The application of these methods to the future study of brucellosis in man will undoubtedly clear up many obscure clinical problems and add still more to the increasing understanding of this now widespread infection.

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—R. A. K.

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## CLINICAL AND EXPERIMENTAL

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### THE FRIEDMAN RABBIT OVULATION TEST IN DIFFERENTIAL OBSTETRIC DIAGNOSIS\*

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ONE of the notable recent advances in medicine has been the development of the biologic tests for pregnancy based on the fundamental discoveries of Smith, Evans, Long, Simpson, Aschheim, Zondek, and others. In this country the original Aschheim-Zondek test has largely been supplanted by the simpler modification of Friedman.<sup>1</sup> The principles and technic are well enough known to need no further description here, but although the results of the original Aschheim-Zondek test have been analyzed by many authors, there has been no recent compilation and discussion of the Friedman test. And yet an understanding of the limitations as well as the value of this biologic method of diagnosing pregnancy is essential for its intelligent use by the many physicians to whom it is now available.

In addition to the new cases added here, the literature through March, 1933, has been thoroughly combed, and every report listed in the *Quarterly Cumulative Index Medicus*, with the exception of a very few in certain foreign languages, carefully analyzed. These have been studied for comparison of technic, percentage accuracy, the cause of false results, and the types of cases in which it has been used. A summary is presented in Table I.

In all, 5,225 cases have been presented by 34 authors, but for analysis sufficient details are given of only 4,515. The gross, uncorrected error is 3.9 per

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cent. The false negative findings have been divided into three sorts: 82 due to limitations of the test, 13 due to technical flaws admitted by the authors, and 54 that cannot be explained. The latter group, together with the 26 false positive findings, amount to 1.8 per cent.

A positive reaction is expected if a suitable rabbit is injected with a quantity of urine containing a sufficient amount of the prehypophyseal hormone. This hormone is generally considered to be elaborated with explosive suddenness shortly after fecundation. Positive tests have been reported as early as ten or twenty days after the known fruitful intercourse. In the author's series the earliest was in a woman with great irregularity of menses, who delivered a seven-pound child 260 days after the test was made. On the other hand, at least 48 cases have been reported (including 3 of this series) where early tests were negative, while later tests have proved correctly positive. The explanation may be that the prehypophyseal stimulation did not occur to the usual extent or else the eliminative threshold of the kidney was different than that usually found. In 7 cases reported, the test remained negative until the fourth or sixth month. Hence, the first limitation of the correctly performed test is that *a negative reading does not rule out early pregnancy.*

TABLE I  
SUMMARY OF CASES REPORTED IN THE LITERATURE

| AUTHOR                          | TECHNIC<br>(SEE TEXT) | NUMBER<br>CASES | NUMBER<br>FALSE<br>POSITIVES | GROSS PERCENTAGE<br>INCORRECT<br>READINGS |
|---------------------------------|-----------------------|-----------------|------------------------------|-------------------------------------------|
| Friedman-Lapham <sup>2</sup>    | FL                    | 92              | 0                            | 0                                         |
| Martins <sup>3</sup>            |                       | 100             |                              |                                           |
| Magath-Randall <sup>4</sup>     | MR                    | 85              | 1                            | 2.4%                                      |
| Beasley <sup>5</sup>            |                       | 45              | 0                            | 2.2%                                      |
| Rhamy <sup>6</sup>              | FL(24 hr.)            | 56              | 0                            | 1.8%                                      |
| Dorn, et al. <sup>7</sup>       | S                     | 120             | 0                            | 2.5%                                      |
| Wilson-Corner <sup>8</sup>      | WC                    | 198             | 1                            | 3.5%                                      |
| White-Severance <sup>9</sup>    | FL                    | 40              | 0                            | 10.0%                                     |
| A. Brouha <sup>10</sup>         | B                     | 171             | 0                            | 3.5%                                      |
| Brindeau-Hinglais <sup>11</sup> | B                     | 32              | 0                            | 10.0%                                     |
| Davis <sup>12</sup>             | S                     | 200             | 4                            | 3.0%                                      |
| Stricker <sup>13</sup>          | RS                    | 75              | 0                            | 0                                         |
| Grant, et al. <sup>14</sup>     | RS                    | 100             | 1                            | 4.0%                                      |
| Mathieu, et al. <sup>15</sup>   |                       | 110             |                              |                                           |
| Wood <sup>16</sup>              | W                     | 95              | 0                            | 3.2%                                      |
| Kineaid-Rafn <sup>17</sup>      | MR                    | 45              | 3                            | 13.0%                                     |
| Schoeneck <sup>18</sup>         | RS-MR-S               | 123             | 0                            | 13.0%                                     |
| Parache <sup>19</sup>           | FL                    | 180             | 0                            | 0.6%                                      |
| Gustafson-Banks <sup>20</sup>   |                       | 50              | 0                            | 16.0%                                     |
| Schneider <sup>21</sup>         | S                     | 500             | 0                            | 0.4 plus %                                |
| Buettner <sup>22</sup>          | S                     | 100             | 2 ?                          | 3.0%                                      |
| Todd <sup>23</sup>              | W (mod)               | 100             | 0                            | 1.0%                                      |
| Russum <sup>24</sup>            | MR                    | 40              | 1                            | 10.0%                                     |
| Sharp-Fluhr <sup>25</sup>       |                       | 73              | 1                            | 1.4%                                      |
| Wilson-Blanchet <sup>26</sup>   | S-FL                  | 250             | 2                            | 1.0%                                      |
| Ware-Main <sup>27</sup>         | S                     | 100             | 0                            | 2.0%                                      |
| Johnson-Townsend <sup>28</sup>  | RS                    | 24              | 1                            | 16.0%                                     |
| Reinhart <sup>29</sup>          | RS                    | 885             | 5                            | 2.3%                                      |
| Sondern-Silverman <sup>30</sup> | S                     | 487             | 0                            | 6.7%                                      |
| Sharpley <sup>31</sup>          | S (mod)               | 38              | 0                            | 0                                         |
| Litterer <sup>32</sup>          | FL                    | 140             | 1                            | 2.7%                                      |
| Mann, et al. <sup>33</sup>      | FL                    | 150             | 2                            | 2.0%                                      |
| King                            | RS-S                  | 86              | 1                            | 8.2%                                      |

In the differential diagnosis between *simple amenorrhea* and *pregnancy*, the author's series includes 40 cases, in all but 4 of which clinical diagnosis was difficult. There were three false negative responses at seven, twelve, and sixty days after the missed period, all later positive. There were 16 correct negative and 21 correct positive diagnoses, including two decisions between subinvolution and early pregnancy before the resumption of menses.

Once pregnancy is established the test will remain positive only as long as live chorionic tissue is in biologic contact with the mother. This must be borne in mind in using the test to diagnose abortion or intrauterine death. There are 107 such cases available for comparison. Of 14 *threatened abortions*, all gave positive reactions except 2 of the author's 5 cases. Both of these began bleeding at three months' gestation; from 5 to 8 c.c. of urine were used and the tests were read in twenty-four hours as negative; in both the bleeding was controlled and the pregnancy continued, positive readings being obtained subsequently. It is suggested that a sudden diminution in the amount of prehypophyseal hormone may have accounted for both the threatened abortion and the negative reactions.

Of *incomplete abortions* the literature describes 37, of which 20 gave positive reactions and 17, negative, in the latter group falling the author's 5 cases. Unfortunately the condition of the placenta is not stated in most of the reports. Two of the cases in the present series deserve some mention; one was seven months postpartum, the other twelve months, and each had bled for some two months previously. The tests were negative, and the pathologist's reports were "placental polyp" and "infected products of conception." Whether these women became pregnant subsequent to their deliveries and then aborted is not known.

In *complete abortion*, of which there are 39 cases, the same conditions hold which apply to the puerperium, namely that from twenty-four to seventy-two hours after delivery either a negative or a positive response may be expected.

*Missed abortion* may be divided into those before the fourth month and those after. Of 8 early cases in the literature, including one of the author's, all gave negative responses. After the fourth month death of the fetus may take place independent of damage to the placenta or its hormone capacity, and hence it is not surprising that Wilson and Corner<sup>8</sup> and Schoeneck<sup>18</sup> each report two positive reactions. A. Brouha<sup>10</sup> obtained two negative reactions, and Ware and Main<sup>27</sup> one. The author has four to add. In three, fetal death between the sixth and seventh month was diagnosed clinically, but positive reactions were obtained seven, seventeen, and thirty days, respectively, after the cessation of fetal movements. In the fourth case a five months' fetus had been dead three months and the rabbit response to the urine was negative. Thus a second limitation of the test is that a *positive reaction does not rule out death of the fetus*.

In *ectopic pregnancy* the test again must be used with discrimination. A positive reading indicates that live chorionic tissue has been in existence within the last seventy-two hours; a negative test means little. Of 57 ectopic pregnancies reported, only 41 gave positive reactions, of which 2 are added in this series. It may be assumed that in the others either the hormone concentration was not sufficient or else the chorionic tissue had been long dead.

On the other hand, in hydatid mole and chorionepithelioma perfect satisfaction is expressed in all reports.

*Cancer* of the uterus, while occasionally giving a positive Aschheim-Zondek test, is usually reported as giving a negative Friedman reaction. One case in this series was negative. But another woman, aged thirty-eight, who had had her last child four years previously and had been bleeding irregularly for two years, passed some tissue, which from description might have been a mole. Two weeks later her Friedman test was positive. She was curetted, and the removed tissue diagnosed by the pathologist as "acanthoma" (squamous cell carcinoma). Two weeks later the Friedman test was again positive. She had a stormy convalescence but recovered, and two months later appeared to be in fair health. This case is presented as the only false positive in the series. The literature includes two other cases of carcinoma which gave positive, although atypical, responses.

In patients of obese habitus it is often difficult or almost impossible to differentiate *fibroids* or *ovarian cyst* from *pregnancy*, and where amenorrhea supervenes the Friedman test becomes particularly valuable. In this series there were 13 such cases of amenorrhea where the physician was frankly doubtful of the diagnosis and the test proved subsequently to be correct: 4 at two months, all negative; 5 at three months of which 1 was a case of pregnancy; and 4 at four months of which 2 were cases of pregnancy.

Of the greatest importance is learning of the existence of *pregnancy* in the presence of a *fibroid* or an *ovarian cyst* before laparotomy. In 6 cases in this series amenorrhea led the gynecologist to use the Friedman test before operating; four were correctly negative, and two correctly positive. In one of these two cases hysterectomy for fibroids was postponed until term; in the other removal of a dermoid cyst was not done until pregnancy had advanced to a point where the safety of the fetus was not threatened.

Other differential diagnoses in this series consisted of: menopause against pregnancy, pelvic inflammatory disease against pregnancy, and finally a case of menorrhagia in a girl of 18 (with a negative test) later shown to be puberty hyperplasia. Table II summarizes the cases in this series in which, with a few exceptions, the test was used to aid the author or other physicians to decide between several possible diagnoses.

It must be borne in mind that many of the false results may have been due to the method of performing the test. Seven different technics with minor variations are described in the literature. These are: that of Friedman and Lapham<sup>2</sup> ("FL"), where 5 c.c. of urine are injected three times a day for two days and the ovaries examined in forty-eight hours; that of Magath and Randall<sup>4</sup> ("MR") who give 15 c.c. and operate in thirty hours; that of Reinhart and Scott<sup>20</sup> ("RS") where a single injection of 5 c.c. is followed by examination in twenty-four hours; that of Wilson and Corner<sup>8</sup> ("WC") who laparotomize the animal in sixteen hours after a 5 c.c. injection; that of Schneider<sup>21</sup> ("S") who uses a larger dosage and waits forty-eight hours; that of A. Brouha<sup>10</sup> ("B") who opens the rabbit before injecting 5 c.c. and makes a final reading in twenty-four hours; and that of Wood<sup>16</sup> ("W") who laparotomizes first, gives 10 c.c., laparotomizes in twenty-four hours, and if negative gives another 10 c.c. and makes a final reading the next day.



The experience of men working with hormone reactions in lower animals indicates that frequent small injections of endocrine substances is far more effective than a single large dose. Friedman and Lapham's 92 cases without an error tends to confirm this observation. While Wilson and Corner obtained excellent results with sixteen-hour readings, many workers including the author have found that the reaction frequently does not become positive until between the twenty-fourth and forty-eighth hour. It is for this reason that although Reinhard and Scott's technic was used at first, in the latter cases of this series 10 or 12 c.c. in a single injection was followed by laparotomy in forty-eight hours as the most desirable routine.

TABLE II

SUMMARY OF 103 TESTS ON 86 WOMEN FOR DIAGNOSIS OF CHORIONIC TISSUE

|                                       | CASES    | POSITIVE | NEGATIVE |
|---------------------------------------|----------|----------|----------|
| Pregnancy vs.<br>Amenorrhea           | 24<br>16 | 21<br>0  | 3<br>16  |
| Threatened abortion                   | 5        | 3        | 2        |
| Incomplete abortion                   | 5        | 0        | 5        |
| Early fetal death                     | 1        | 0        | 1        |
| Later fetal death                     | 4        | 3        | 1        |
| Ectopic pregnancy                     | 2        | 2        | 0        |
| Cancer                                | 2        | 1        | 1        |
| Fibroids or cyst<br>vs. pregnancy     | 12<br>3  | 0<br>3   | 12<br>0  |
| Tumors with, vs.<br>without pregnancy | 2<br>4   | 2<br>0   | 0<br>4   |
| Menopause                             | 3        | 0        | 3        |
| P.I.D.                                | 2        | 0        | 2        |
| Menorrhagia                           | 1        | 0        | 1        |

It is not unlikely that the breed of rabbit and the climate or season determines whether or not ovulation is quickly produced. The size of the rabbit is of equal importance. The lower limits as given by most authors are 1.5 kg., ordinarily three and one-half months of age. Adult rabbits are widely used after proper isolation even from other females. While Davis<sup>12</sup> considers fourteen days sufficient (but reports 4 false positive reactions) twenty-one to twenty-eight days is usually held to be the minimum. Death of the rabbit from a toxic factor in the urine is reported by six different authors as occurring in 2.7 per cent, 2.7 per cent, 5.8 per cent, 5.8 per cent (this series), 8.9 per cent, and 12 per cent. The cause of death, be it too acid or too cold a urine, or some poisonous substance has not been determined. Simple anaphylaxis, however, seems to have been ruled out.

As Snyder and Wislocki<sup>24</sup> have pointed out, the Friedman test is specific for the human female, and hence is not applicable to veterinary purposes. Ignorance of this fact recently led a well-known gynecologist to condemn the test in general for its failure to indicate pregnancy in three of his mares. On the other

hand the work of White and Severance<sup>9</sup> shows that in other respects the test is not as delicate as that of L. Brouha on male mice.

Finally there must be considered what constitutes a positive reaction. It is agreed that a translucent follicle, without color, is a negative response. Wilson and Corner<sup>3</sup> using a sixteen-hour technic describe a freshly ruptured, bleeding, red follicle as a true positive, and warn against a small, dark brown, rather conical body occasionally seen. Most authors look for a large, rounded, purplish corpus hemorrhagicum. Buettner<sup>22</sup> reports as a true positive a large pale unruptured follicle with tiny red flecks. Laparotomy within a week of three such observations showed the structures to have formed true corpora lutea. Grant<sup>14</sup> also observed such a follicle in one case which he reported as positive. Definite decision can be made by microscopic examination which will reveal beginning luteinization. Gustafson and Banks<sup>20</sup> report 5 cases grossly negative but correctly positive after histologic study. In the author's series a similar instance is recorded.

The cause of false positive responses is still to be worked out. Table III summarizes the false positive cases according to the cause or the diagnosis. Aside from the technical errors in handling the rabbits, any condition which might cause stimulation of the anterior lobe of the pituitary gland might be expected to cause a development of *prolan* which may be reflected in a positive rabbit reaction. Kraus<sup>35</sup> reports that an increase in *prolan* may be the result of elevated intracranial pressure. Germ cell tumors, pituitary gland tumors, compensatory hypertrophy of the pituitary gland, must all be considered.

The Friedman test for the diagnosis of pregnancy or its products is rapidly becoming a part of the physician's equipment along with the Wassermann test, agglutination reactions, and the leucocyte count. But it must retain its proper relationship to history taking and physical diagnosis. An interesting experience of one of the leading gynecologists of the country emphasizes its usefulness: his

TABLE III  
TABULATION OF FALSE POSITIVE REACTIONS

|                      |   |                   |
|----------------------|---|-------------------|
| Details not given    | 9 |                   |
| Uncontrolled rabbit  | 2 |                   |
| Improper segregation | 2 |                   |
| Puberty bleeding     | 1 | Wilson and Corner |
| Castration           | 1 | Sharp-Flohr       |
| Teratoma testis      | 1 | Russum            |
| Carcinoma            | 3 | Grant, et al.     |
|                      |   | Buettner          |
|                      |   | King              |
| Unexplained          | 7 |                   |

diagnosis of a case was that of a prolapsed twisted ovarian cyst, while his assistant was convinced that the patient had a recently ruptured ectopic pregnancy. The Friedman test was positive; laparotomy revealed on one side the tubal pregnancy and on the other side a small cyst which had prolapsed into the culdesac and which he had felt. But on the other hand, teachers in all branches of medicine find that their students, instead of training themselves to detect

pregnancy by their five senses, depend on a rabbit test to make the diagnosis. This test, however valuable, should never become popular at the expense of the *Tactus cruditus*.

### CONCLUSIONS

1. The results of 103 Friedman rabbit ovulation tests on 86 women are reported and discussed.
2. The literature to date is reviewed. Of 4,500 cases, the gross error has been 3.9 per cent; the corrected error, including the 26 false positive readings, amounts to 1.8 per cent.
3. The test is a valuable aid in differential diagnosis, provided its limitations are recognized.
4. A positive test indicates usually, but not always, the presence of live chorionic tissue. It may not become positive until pregnancy is well advanced, and if positive may not necessarily mean that the fetus is alive.
5. The test should assist but not supplant the usual diagnostic technic.

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1430 TULANE AVENUE

## THE RELATIONSHIP BETWEEN THE CORONARY BLOOD SUPPLY TO EXPERIMENTALLY PRODUCED VENTRICULAR LESIONS AND THE RESULTING ELECTROCARDIOGRAPHIC ALTERATIONS\*

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IN A RECENT publication<sup>1</sup> the authors reported the electrocardiographic changes following ventricular myocardial lesions produced by the electric cautery. Particular attention was directed to the R-T segment and T-wave alterations resulting from definitely placed lesions, the localization of which was related to certain electrocardiographic characteristics as classified by Parkinson and Bedford.<sup>2</sup> It was found that cauterization of the anterior surface of the left ventricle gave curves of the T<sub>1</sub> type while a similar lesion of the posterior surface of this ventricle and of any portion of the right ventricle (except at the base anterior where no change was observed) resulted in curves of the T<sub>2</sub> type. Substantially similar results have been obtained recently by Haney, Borman and Meek<sup>3</sup> who investigated the changes in the R-T and S-T segments of the electrocardiogram in dogs in which the myocardial lesions were produced by radon implants.

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This report is concerned with the relationship between the blood supply (as from the right or left coronary artery) to well-defined ventricular areas damaged by the electric cautery and the resulting electrocardiographic alterations. In a series of twelve cats, the coronary arteries were injected, by a method previously described,<sup>4</sup> and the vessel distribution, with respect to the damaged areas, studied in detail.

TABLE I

COMPARISON BETWEEN THE SITE BURNED, THE TYPE OF CURVE PRODUCED AND THE BLOOD SUPPLY

| NO. | SITE BURNED      | TYPE           | LEFT CORONARY | RIGHT CORONARY | MOSTLY LEFT CORONARY | MOSTLY RIGHT CORONARY |
|-----|------------------|----------------|---------------|----------------|----------------------|-----------------------|
| 3   | Left apex ant.   | T <sub>1</sub> | +             |                |                      |                       |
| 6   | Left apex post.  | T <sub>2</sub> | +             |                |                      |                       |
| 7   | Left base post.  | T <sub>2</sub> |               |                |                      | +                     |
| 30  | Left apex ant.   | T <sub>1</sub> | +             |                |                      |                       |
| 31  | Left apex post.  | T <sub>2</sub> |               |                | +                    |                       |
| 32  | Right apex ant.  | T <sub>2</sub> |               |                | +                    |                       |
| 33  | Left base post.  | T <sub>2</sub> |               |                | +                    |                       |
| 34  | Left apex post.  | T <sub>2</sub> | +             |                |                      |                       |
| 35  | Left base post.  | T <sub>2</sub> | $\frac{1}{2}$ | $\frac{1}{2}$  |                      |                       |
| 36  | Right apex post. | T <sub>2</sub> |               |                | +                    |                       |
| 37  | Right base post. | T <sub>2</sub> |               |                |                      | +                     |
| 38  | Right base post. | T <sub>2</sub> |               | +              |                      |                       |

RESULTS (TABLE I)

*Anterior Surface of the Left Ventricle.*—Two specimens (No. 3 and No. 30) were studied, in both of which the lesion had been produced at the apex and a T<sub>1</sub> type of curve obtained. In each instance the whole of the damaged area was supplied by the left coronary artery.

*Posterior Surface of the Left Ventricle.*—Six hearts, in all of which the resulting curve had been of T<sub>2</sub> type, were injected. In three the lesion was at the apex. Of these the left coronary artery supplied the whole of the injured area in two (No. 6 and No. 34) and the greater part of it in the third (No. 31). In the other three, the damage was at the base, the left coronary being the principal supply in one (No. 33), the right coronary in another (No. 7), while both played an equal part in the third (No. 35).

*Anterior Surface of the Right Ventricle.*—One heart (No. 32), in which a lesion had been produced at the apex and a T<sub>2</sub> type of curve obtained, was studied. The left coronary artery was the principal supply to this area.

*Posterior Surface of the Right Ventricle.*—This series consisted of three hearts, in which all of the curves were of the T<sub>2</sub> type. In one instance (No. 36), the damaged area was situated at the apex with the left coronary artery as the chief source of supply. In the other two, in which the lesions were at the base, the right coronary artery supplied the whole region in one (No. 38) and the greater part of it in the other (No. 37).

To summarize: It was found that in the two experiments in which a T<sub>1</sub> type of curve was obtained, the damaged site was supplied in whole by the left coronary artery. In the instances in which a T<sub>2</sub> type of curve resulted, the coronary distribution varied considerably. The area involved was sup-

plied wholly by the left coronary artery in two cases and in great part in four others. In the remainder, the right coronary artery supplied all of the cauterized region in one, was the main supply in two, while in another both vessels appeared to be of equal importance.

#### DISCUSSION

Barnes and Whitten<sup>5</sup> found that in infarcts of the anterior portion of the left ventricle, either alone or combined with a similar lesion at the apex, curves of the  $T_1$  type were produced; whereas in those of the posterior surface, with or without involvement at the apex, the tracings were of the  $T_2$  type. They believed that it was probably more than a coincidence that the plane of division which decided the type of electrocardiogram should follow the average plane of separation between the blood supply from the right and left coronary arteries. Despite this they suggested that it was the site of the lesion rather than the particular blood supply which determined the changes in the R-T interval, but that the site would usually indicate the artery involved. Bell and Pardee<sup>6</sup> analyzed five cases of their own and seven of Levine's series<sup>7</sup> and found that thrombosis of the left coronary artery was associated with changes of  $T_1$  type, while occlusion of the right coronary artery gave tracings of  $T_2$  type. They stated: "We therefore feel that in the absence of bundle-branch block, the electrocardiogram may be used in localizing the thrombus in either the right or left coronary system. We cannot offer any particular explanation for this relation between the vessel occluded and the particular T-wave that is inverted." On the other hand, Gilchrist and Ritchie,<sup>8</sup> as a result of a study of two cases of their own and a large series from the literature, concluded that changes in the R-T segment were strong presumptive evidence of myocardial infarction, but they did not believe the data available sufficient to warrant the view that the form of the electrocardiographic change could be considered a definite localizing sign of the infarct. Barnes and Mann<sup>9</sup> investigated the question in dogs surviving for varying periods, electrocardiograms being taken at intervals until death, when autopsies were performed. These workers tied off branches of the left coronary artery, supplying the posterior surface of the left ventricle, and obtained  $T_1$  type of curves, ligation of the right coronary artery resulting in  $T_2$  type. When one considers that a  $T_1$  type was obtained when the left ventricle was infarcted and a  $T_2$  type when a similar lesion was produced in the right ventricle, the fact that in the dog the left coronary artery supplies the whole of the left ventricle and the right coronary, nearly the whole of the right<sup>10</sup> might assume some importance in establishing a relationship between the type of RS-T alteration and the blood supply. Despite their findings, these authors do not consider the latter to be the determining factor, since they state that in man the electrocardiographic changes following acute infarction of the posterior basal portion of the left ventricle are constant regardless of whether this region is supplied by the right or left coronary artery. Wilson and his collaborators,<sup>11</sup> on correlating the form of the electrocardiogram with the location of the infarct in the human heart, state that the  $Q_1T_1$  type is usually asso-

ciated with a lesion of the anterior surface of the left ventricle, the  $Q_3T_3$  with one of the posterior surface. In some instances, however, lesions of these sites did not produce any characteristic change in the electrocardiogram. Fowler, Rathe and Smith<sup>12</sup> in a series of survival experiments in dogs, ligated small branches of the coronary arteries, both right and left, and found mainly T-wave changes. They were unable to correlate the location of the infarct and the type of electrocardiogram produced. Our results in the cat show that on the anterior surface of the left ventricle, the blood supply was always from the left coronary artery, and the electrocardiograms, following the production of localized lesions, were of the  $T_1$  type. Over the entire surface of the heart posteriorly,  $T_3$  types of curves were obtained, despite marked variations in blood supply. When one studies the relationship of all the  $T_3$  type of curves to the blood supply of the damaged areas without consideration of the region of the heart involved, the results appear grossly inconsistent. This view is further substantiated by an examination of the normal coronary distribution in the cat. In a recent study dealing with the latter,<sup>4</sup> it was found that posteriorly, in 65 per cent of cases the right coronary artery supplied all of the right ventricle and varying portions of the left ventricle, ranging from a small strip adjoining the posterior interventricular sulcus to almost one-half of the left ventricle. The remainder of this ventricle received its supply from the left coronary artery. In the other 35 per cent of cases the left coronary artery supplied posteriorly all of the left ventricle and the adjoining part of the right ventricle, while the right coronary in these hearts supplied only a small portion of the right ventricle.

In view of these anatomical observations we believe that the coronary distribution in the cat resembles that in man to a greater degree than does that in the dog. Although on the anterior surface of the heart, the subbranches of the right and left coronary arteries show a generally similar arrangement in all three, posteriorly there are some marked differences. In man, according to Gross<sup>13</sup> and also Barnes and Whitten,<sup>5</sup> the right coronary artery in the majority of cases gives off a posterior descending branch which extends down the posterior interventricular sulcus; the right coronary artery, itself, continuing on to the left ventricle to supply a considerable part of the latter adjacent to the sulcus. In these cases the circumflex branch of the left coronary plays an unimportant rôle in supplying the posterior surface of the heart. In a small percentage of cases, the situation is reversed, the circumflex reaching the posterior sulcus and continuing down in it, while the right coronary artery terminates soon after it reaches the posterior surface of the right ventricle. In these instances, posteriorly the whole of the left ventricle and the adjoining portion of the right are supplied by the left coronary artery. In the dog, according to Moore<sup>10</sup> the right coronary artery never reaches the posterior interventricular sulcus, but ends at some point between it and the right border of the heart. In all cases, the circumflex division of the left coronary artery proceeds down the posterior sulcus as the posterior descending division, and along with its other branches, supplies the whole of the left ventricle posteriorly. In man, the septum in its basal two-thirds is supplied by penetrating branches of the anterior and posterior descending arteries, subdivisions usu-

ally of the left and right coronary arteries respectively. The apical portion is supplied entirely by twigs from the anterior descending artery. In the case of the dog, branches of the septal division of the left coronary artery anastomose with penetrating vessels from the anterior and posterior descending arteries, the whole of the septum thereby being supplied by the left coronary artery. In the cat, in the majority of cases, the septum receives its supply from penetrating branches of both right and left coronary arteries, while in the remaining hearts, wholly from branches of the latter.

The R-T segment alterations associated with myocardial damage after blood vessel occlusion or direct injury are, we think, the expression in a limb lead of a monophasic variation of the demarcation current set up in the heart as the result of a potential difference between the injured and the adjacent uninjured areas. One should not expect that thrombosis of a particular coronary artery, per se, could produce the definite change observed in the electrocardiogram in coronary thrombosis. Only in the case of a constant blood supply would a characteristic change in the electrocardiogram be related to the occlusion of a particular artery. In our experiments on the cat, the blood supply (as to right or left coronary arteries) to the posterior surface of the heart varied markedly, and still on cauterization of any portion of this site a  $T_2$  type of curve was obtained in every instance. When we consider as well that the anatomical studies have pointed to a close resemblance between the coronary blood supply in man and that in the cat, it strengthens our belief that in the former it is hazardous to diagnose a thrombus in one or the other coronary vessel in a  $T_2$  type of curve. With a  $T_1$  type there appears to be every probability that the lesion is located in the left coronary artery. The final decision on this question in man must await the careful study of a sufficient number of hearts in which the electrocardiographic and pathologic data have been investigated and correlated. On the other hand, since all authors agree that infarct of the right ventricle is rare, it seems to us probable that a definite  $T_1$  type of electrocardiogram indicates a lesion of the anterior surface of the left ventricle and a  $T_2$  type one of its posterior surface.

#### SUMMARY AND CONCLUSION

The relationship between the blood supply (as from the right or left coronary artery) to experimentally produced ventricular lesions and the resulting electrocardiographic alterations was investigated in a series of injected cats' hearts.

Although on the anterior surface of the heart, there appeared to be a relationship between the curves and the blood supply, on the posterior surface, both of the right and left ventricles, tracings of  $T_2$  type were produced, whereas the blood supply to these areas varied markedly.

The results support the view that the characteristic T-wave changes are not constantly related to the occlusion of a particular coronary artery.

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## THE EFFECT OF HYPERPYREXIA UPON THE PHOSPHORUS PARTITION OF WHOLE BLOOD IN PARESIS\*

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RECENT investigations have shown there are changes in the amount of the various phosphorus fractions of the blood of neurosyphilitic patients following treatment. Rosen, Krasnow, and Notkin<sup>1</sup> have found a distinct increase in the amount of lecithin in the blood of syphilitic patients after treatment. Bischoff, Maxwell, and Hill<sup>2</sup> and Daly and Knudson<sup>3</sup> have demonstrated that there is a shift in the phosphorus equilibrium during hyperthermia.

### PLAN OF EXPERIMENT

The results of the work cited above would indicate that the treatment of paresis with hyperpyrexia might have a definite influence upon the phosphorus partition of the blood. This problem was approached by the following procedure: Fifteen patients diagnosed as typically paretic were chosen for this study; nine were treated by the electric blanket method<sup>4</sup> and six with inoculated tertian malaria. A phosphorus partition, involving inorganic, total acid-soluble, organic acid-soluble, lipin, and total phosphorus, was determined upon the whole blood of each patient before and after the treatment series. Those treated by the electric blanket method each received twelve treatments, and those who were given malaria were each allowed to have five paroxysms.

\*From the Illinois State Psychopathic Institute.  
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The blood for analysis was taken in the morning after a fifteen-hour fast, and the patients, while undergoing the series of treatments, were kept under carefully controlled hospital conditions.

The methods of analysis used in this study were those recommended by Walker and Huntsinger.<sup>5</sup> Each determination was done in duplicate and checked independently by two workers. Mean values were used when duplicate determinations did not agree.

TABLE I

PHOSPHORUS PARTITION OF WHOLE BLOOD IN PARETIC PATIENTS BEFORE AND AFTER TREATMENT WITH HYPERPYREXIA

| CASE   | 1<br>TOTAL<br>ACID-<br>SOLUBLE<br>P<br>(MG. PER<br>100 ML.) | 2<br>INORGANIC<br>P<br>(MG. PER<br>100 ML.) | 3<br>ORGANIC<br>ACID-<br>SOLUBLE P<br>(1 MINUS 2)<br>(MG. PER<br>100 ML.) | 4<br>LIPIN<br>P<br>(MG. PER<br>100 ML.) | 5<br>TOTAL P<br>BY<br>ANALYSIS<br>(MG. PER<br>100 ML.) | 6<br>TOTAL P<br>BY<br>ADDITION<br>(1 PLUS 4)<br>(MG. PER<br>100 ML.) | CONDITION<br>OF<br>PATIENT |
|--------|-------------------------------------------------------------|---------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------|--------------------------------------------------------|----------------------------------------------------------------------|----------------------------|
| 1      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 21.79                                                       | 3.25                                        | 18.54                                                                     | 10.63                                   | 33.33                                                  | 32.42                                                                |                            |
| After  | 19.61                                                       | 3.65                                        | 15.96                                                                     | 12.04                                   | 33.33                                                  | 31.65                                                                | Improved                   |
| 2      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 26.31                                                       | 2.62                                        | 23.69                                                                     | 11.07                                   | 36.27                                                  | 37.38                                                                |                            |
| After  | 25.00                                                       | 3.27                                        | 21.73                                                                     | 12.20                                   | 36.86                                                  | 37.20                                                                | Improved                   |
| 3      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 23.88                                                       | 1.63                                        | 22.25                                                                     | 11.33                                   | 35.02                                                  | 35.21                                                                |                            |
| After  | 20.20                                                       | 3.50                                        | 16.70                                                                     | 11.87                                   | 32.56                                                  | 32.07                                                                | Improved                   |
| 4      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 22.47                                                       | 2.66                                        | 19.81                                                                     | 12.15                                   | 31.37                                                  | 34.62                                                                | Not                        |
| After  | 20.72                                                       | 3.12                                        | 17.60                                                                     | 11.87                                   | 32.56                                                  | 32.59                                                                | Improved                   |
| 5      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 24.84                                                       | 3.32                                        | 21.52                                                                     | 12.87                                   | 35.49                                                  | 37.71                                                                | Not                        |
| After  | 20.44                                                       | 2.53                                        | 17.91                                                                     | 12.12                                   | 32.26                                                  | 32.56                                                                | Improved                   |
| 6      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 25.71                                                       | 3.26                                        | 22.45                                                                     | 11.43                                   | 34.84                                                  | 37.14                                                                |                            |
| After  | 21.62                                                       | 2.54                                        | 19.08                                                                     | 12.05                                   | 34.04                                                  | 33.67                                                                | Improved                   |
| 7      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 26.49                                                       | 3.88                                        | 22.61                                                                     | 13.97                                   | 40.81                                                  | 40.46                                                                |                            |
| After  | 27.58                                                       | 3.20                                        | 24.38                                                                     | 14.81                                   | 42.55                                                  | 42.39                                                                | Improved                   |
| 8      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 26.14                                                       | 3.24                                        | 22.90                                                                     | 12.00                                   | 36.36                                                  | 38.11                                                                |                            |
| After  | 30.76                                                       | 2.92                                        | 27.84                                                                     | 14.13                                   | 43.71                                                  | 44.89                                                                | Improved                   |
| 9      |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 23.81                                                       | 3.46                                        | 20.35                                                                     | 10.52                                   | 34.93                                                  | 34.33                                                                |                            |
| After  | 23.53                                                       | 2.96                                        | 20.57                                                                     | 12.87                                   | 36.03                                                  | 36.40                                                                | Improved                   |
| 10     |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 25.62                                                       | 3.15                                        | 22.47                                                                     | 14.30                                   | 40.55                                                  | 39.92                                                                |                            |
| After  | 24.07                                                       | 2.90                                        | 21.17                                                                     | 14.81                                   | 39.02                                                  | 38.88                                                                | Improved                   |
| 11     |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 29.62                                                       | 3.11                                        | 26.51                                                                     | 13.11                                   | 42.23                                                  | 42.73                                                                |                            |
| After  | 25.74                                                       | 2.81                                        | 22.93                                                                     | 14.61                                   | 39.02                                                  | 40.35                                                                | Improved                   |
| 12     |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 30.01                                                       | 3.72                                        | 26.29                                                                     | 12.69                                   | 42.78                                                  | 42.70                                                                |                            |
| After  | 24.84                                                       | 3.91                                        | 20.93                                                                     | 14.29                                   | 38.64                                                  | 39.13                                                                | Improved                   |
| 13     |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 28.98                                                       | 3.65                                        | 25.33                                                                     | 13.33                                   | 42.75                                                  | 42.31                                                                |                            |
| After  | 23.52                                                       | 2.87                                        | 20.65                                                                     | 14.54                                   | 37.20                                                  | 38.06                                                                | Improved                   |
| 14     |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 29.19                                                       | 3.90                                        | 25.29                                                                     | 14.35                                   | 43.24                                                  | 43.54                                                                |                            |
| After  | 27.58                                                       | 3.55                                        | 24.03                                                                     | 15.50                                   | 43.53                                                  | 43.08                                                                | Improved                   |
| 15     |                                                             |                                             |                                                                           |                                         |                                                        |                                                                      |                            |
| Before | 23.12                                                       | 3.63                                        | 19.49                                                                     | 14.44                                   | 38.08                                                  | 37.56                                                                |                            |
| After  | 26.13                                                       | 3.90                                        | 22.23                                                                     | 15.09                                   | 40.00                                                  | 41.22                                                                | Improved                   |

## RESULTS

The data incorporated in Table I give the findings of the various phosphorus partitions before and after treatment. The accuracy of the analytic methods employed is demonstrated by the close agreement between the total phosphorus by direct analysis and the sum of the acid-soluble and lipin fractions. This table also gives the status of each patient following treatment.

Table II gives the amount of lecithin per 100 ml. of whole blood for each patient before and after treatment. These figures were obtained by multiplying

TABLE II  
LECITHIN CONTENT OF WHOLE BLOOD IN PARETIC PATIENTS BEFORE AND AFTER TREATMENT  
WITH HYPERPYREXIA

| CASE | BEFORE<br>(MG. PER 100 ML.) | AFTER<br>(MG. PER 100 ML.) | CONDITION OF PATIENT<br>AFTER TREATMENT |
|------|-----------------------------|----------------------------|-----------------------------------------|
| 1    | 265.75                      | 301.00                     | Improved                                |
| 2    | 276.75                      | 305.00                     | Improved                                |
| 3    | 283.25                      | 296.75                     | Improved                                |
| 4    | 303.75                      | 296.75                     | Not improved                            |
| 5    | 321.75                      | 303.00                     | Not improved                            |
| 6    | 285.75                      | 301.25                     | Improved                                |
| 7    | 339.15                      | 370.25                     | Improved                                |
| 8    | 300.00                      | 353.25                     | Improved                                |
| 9    | 263.00                      | 321.75                     | Improved                                |
| 10   | 357.50                      | 370.25                     | Improved                                |
| 11   | 327.75                      | 365.25                     | Improved                                |
| 12   | 317.25                      | 357.25                     | Improved                                |
| 13   | 333.25                      | 363.50                     | Improved                                |
| 14   | 358.75                      | 387.50                     | Improved                                |
| 15   | 361.00                      | 377.25                     | Improved                                |

the lipin phosphorus by 25, as lecithin contains 4 per cent phosphorus. This gives recognition only to the lecithin content of this fraction, but this has been found accurate enough for comparative purposes. The status of each patient following treatment is also included in this table.

## DISCUSSION

Bischoff et al.<sup>2</sup> have found that during hyperthermia part of the inorganic phosphorus in the blood is converted into acid-soluble organic phosphorus with an alkaline shift in the blood  $P_H$ . They have suggested that this is an in-vivo duplication of the in-vitro conversion of inorganic to organic phosphorus in blood as first demonstrated by Lawaczek.<sup>6</sup>

Daly and Knudson<sup>3</sup> in their experiment in hyperthermia were unable to confirm the observation of Bischoff and his coworkers that the alkalosis occurring during hyperthermia was accompanied by an increase in the acid-soluble organic phosphorus and a decrease in the inorganic fraction. They found that there was a lowering of both inorganic and acid-soluble organic phosphorus of whole blood and plasma during alkalosis.

It was also shown by Rosen et al.<sup>1</sup> that patients with neurosyphilis, who improve following treatment, show an increase in the lecithin content of their blood.

Referring to Table I and excluding patients 4 and 5, who did not improve following treatment, a lowering of the inorganic phosphorus content is shown in eight of the thirteen improved cases. Again nine of these thirteen patients had a loss of organic acid-soluble phosphorus. This table also shows that all of the thirteen improved cases had an increase in the amount of the lipin phosphorus fraction.

The above facts are not in perfect agreement with the findings of either Bischoff or Daly and Knudson. This perhaps can be explained by the fact that their analyses were done during the period of hyperthermia while samples analyzed in this problem were taken at the normal body temperature before and after the treatment series. However, the results of the majority of our analyses agreed with the findings of Daly.

There is evidence that the synthesis of phosphatides from inorganic phosphates can take place in the body.<sup>7</sup> Animals have been shown to develop normally when their only source of phosphorus was in the inorganic form, and to be capable of forming both organic phosphorus for their bodies and lecithin for their eggs. It is significant to note, therefore, that in nine of the thirteen improved cases there was an increase in the lipin fraction accompanied by a lowering of both the total acid-soluble and organic acid-soluble phosphorus. This might be used as evidence that part of the acid-soluble fraction had been converted into lipin phosphorus as a result of the hyperpyrexia.

Regarding the total determined phosphorus in Table I, it will be noticed that there were no significant changes in the amount following treatment in the thirteen improved cases. It increased in five, decreased in seven and remained constant in one. This would suggest that the increase in the lipin phosphorus was not caused by an increase in the total amount of phosphorus in the blood, but represented only a shift from one fraction to another.

The only significant change observed in the unimproved Cases 4 and 5 was that there was a decrease in the amount of lipin phosphorus after treatment. This may prove to be indicative, but two cases can hardly be considered as sufficient evidence to arrive at any definite conclusion.

Table II shows that there was an increase in the lecithin content of the blood of the improved cases. This agrees with the findings of Rosen et al.<sup>1</sup>

#### CONCLUSIONS

1. Parietic patients who improved following treatment by hyperpyrexia showed an increase in the lecithin content of their blood.
2. There seemed to be a conversion of acid-soluble phosphorus to lipin phosphorus following treatment by hyperpyrexia in those cases which improved.
3. The increase in the amount of lipin phosphorus in the blood of improved patients following hyperpyrexia was not accompanied by a corresponding increase in the total determined phosphorus, and would seem to be a partitional change.

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## SPINAL FLUID SUGAR DETERMINATIONS IN EXPERIMENTAL HYPOGLYCEMIA OF DOGS\*

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RECENTLY one of us (R. D.) had occasion to treat an unusual case of severe insulin shock. The patient, a man aged thirty-one years, was a known diabetic patient and, while under the influence of alcohol, took 30 units of insulin before the evening meal, which was twice his prescribed dose. He promptly vomited and members of his family thinking he was going into a diabetic coma administered two more 30-unit doses of insulin. The patient became more stuporous during the night and was sent to the hospital in the morning.

On admission his blood sugar was 21 mg. per 100 c.c. A total of 100 gm. of glucose was given intravenously, but the patient did not regain consciousness though the blood sugar was then 164 mg. per 100 c.c. Intravenous injections of glucose were continued but the patient died without regaining consciousness. The blood sugar after death was 176 mg. per 100 c.c., but the spinal fluid sugar was only 25 mg. per 100 c.c.

It is difficult to evaluate the rôle played by alcohol in this case but the discrepancy between blood and spinal fluid sugar results made it desirable that a study be made of the blood and spinal fluid sugar in experimental hypoglycemia in the hope that the poor response of such patients to large amounts of injected glucose might be explained.

### EXPERIMENTAL

Normal dogs of both sexes and various weights were used. Insulin was administered after a twenty-four-hour fasting period. Blood was taken from the jugular vein and spinal fluid obtained by cisternal puncture without anesthesia. The specimens were preserved with sodium fluoride and the glucose determinations made by the method of Folin and Wu.<sup>1</sup>

Table I lists the blood and spinal fluid sugar findings of five normal dogs. It is generally agreed that in a state of health the blood and spinal fluid sugar are in a fairly definite relationship to each other. However, we are unaware of

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any simultaneous determinations made on the experimental animal. Kasahara and Uetani<sup>2</sup> though giving figures for spinal fluid sugar following insulin injections did not report simultaneous blood sugar values, and the relationship of the two values were only assumed. In humans this ratio is approximately 4:3 which corresponds closely to what we have found in dogs.

TABLE I  
BLOOD AND SPINAL FLUID SUGAR OF NORMAL DOGS

| DOG NO. | BLOOD SUGAR<br>MG. PER<br>100 C.C. | SPINAL FLUID<br>SUGAR MG. PER<br>100 C.C. | RATIO |
|---------|------------------------------------|-------------------------------------------|-------|
| 1       | 91                                 | 68                                        | 1.34  |
| 2       | 84                                 | 65                                        | 1.29  |
| 3       | 81                                 | 60                                        | 1.35  |
| 4       | 108                                | 73                                        | 1.48  |
| 5       | 82                                 | 64                                        | 1.28  |
| Average | —                                  | —                                         | 1.35  |

TABLE II  
EFFECT OF INSULIN HYPOGLYCEMIA ON SPINAL FLUID SUGAR

| DOG NO.       | FASTING<br>BLOOD<br>SUGAR<br>(MG. PER<br>100 C.C.) | FASTING<br>SPINAL<br>FLUID<br>SUGAR<br>(MG. PER<br>100 C.C.) | INSULIN                                                                                                                                   | "SHOCK"<br>BLOOD<br>SUGAR<br>(MG. PER<br>100 C.C.) | "SHOCK"<br>SPINAL<br>FLUID<br>SUGAR<br>(MG. PER<br>100 C.C.) | RATIO |
|---------------|----------------------------------------------------|--------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|--------------------------------------------------------------|-------|
| 1<br>8 kg.    | 11:20 A.M.<br>91                                   | 11:20 A.M.<br>68                                             | 11:30 A.M.<br>80 units<br>(subcutaneously)                                                                                                | 1:40 P.M.<br>25                                    | 1:45 P.M.<br>24                                              | 1.04  |
| 2<br>11 kg.   | 10:40 A.M.<br>84                                   | 10:40 A.M.<br>65                                             | 10:55 A.M.<br>110 units<br>(subcutaneously)                                                                                               | 12:55 P.M.<br>37                                   | 1 P.M.<br>23                                                 | 1.61  |
| 3<br>10.4 kg. | 4:15 P.M.<br>82                                    | 4:20 P.M.<br>64                                              | 4:25 P.M.<br>20 units<br>(subcutaneously)                                                                                                 | 6 P.M.<br>44                                       | 6:05 P.M.<br>39                                              | 1.14  |
| 4<br>11.8 kg. | 3:40 P.M.<br>108                                   | 3:55 P.M.<br>73                                              | 4 P.M.<br>59 units<br>(subcutaneously)                                                                                                    | 5:30 P.M.<br>51                                    | 5:35 P.M.<br>41                                              | 1.24  |
| 5<br>11.8 kg. | 1:20 P.M.<br>81                                    | 1:28 P.M.<br>61                                              | 1:30 P.M.<br>59 units<br>(intraspinally)                                                                                                  | 3:05 P.M.<br>61                                    | 3:15 P.M.<br>32                                              | 1.91  |
| 6<br>10.3 kg. | —                                                  | —                                                            | 11:45 A.M.<br>60 units<br>(subcutaneously)                                                                                                | 1:15 P.M.<br>34                                    | 1:20 P.M.<br>28                                              | 1.21  |
| 7<br>10.2 kg. | —                                                  | —                                                            | 9:55 A.M.<br>80 units<br>(subcutaneously)                                                                                                 | 11:55 A.M.<br>44                                   | 12 N<br>33                                                   | 1.33  |
| 7<br>10.2 kg. | —                                                  | —                                                            | 10:40 A.M.<br>40 units<br>(intraspinally)                                                                                                 | 12:15 P.M.<br>33                                   | 12:20 P.M.<br>23                                             | 1.44  |
| 8<br>12.6 kg. | —                                                  | —                                                            | 9:55 A.M.<br>40 units<br>11:30 A.M.<br>80 units<br>12:35 P.M.<br>40 units<br>1:20 P.M.<br>40 units<br>(subcutaneously)<br>Total 200 units | 2:10 P.M.<br>33                                    | 2:15 P.M.<br>17                                              | 1.94  |

In the production of hypoglycemia in dogs Drabkin and Shilkret<sup>3</sup> used 20 units of insulin per kilogram. In our work we used smaller doses, not considering it desirable to produce the severe hypoglycemia that is obtained with such large doses. Our dosage ranges were between 2 and 10 units per kilogram with one exception. Dog No. 8 received 15.8 units per kilogram. The criteria of shock were listlessness, apathy, unsteady gait, vomiting, and loss of control of bowels and bladder.

It will be noted from Table II that in no case did the spinal fluid fail to show a decided decrease in sugar following insulin administration. The two dogs receiving insulin intraspinally showed a somewhat greater proportional fall in spinal fluid sugar per unit of insulin but part of this excessive decrease might be accounted for by simple dilution of spinal fluid by the insulin. The blood and spinal fluid sugar relationship is definitely disturbed; in only one animal was the normal ratio maintained. It is also noteworthy that in very severe hypoglycemia (dog No. 8) the effect on the spinal fluid sugar is proportionally so much greater as to cause this ratio to approach two. Insulin is active intraspinally; the blood sugar findings, however, indicate that it is no more efficacious than when administered subcutaneously.

The influence of intraspinal glucose administration upon insulin hypoglycemia is illustrated in Table III.

After shock had been attained in the six dogs, varying amounts of glucose solution were injected intraspinally. Following each injection, the blood sugar, as well as the spinal fluid sugar, was determined in order to see if there was a rise in the blood value coincident with any improvement noted.

Mater<sup>4</sup> has reported a constant rise in the blood sugar following the injection of air and Ringer's solution into the subarachnoid space, amounting to 200 mg. per cent in some cases. Such a rise, however, could be suppressed by the preliminary use of insulin. Our results confirm these observations, the blood sugar rising but little following intraspinal glucose injections in the insulin-treated dogs.

In all our cases of intraspinal glucose injections the improvement noted was transient and difficult to evaluate. To eliminate the possible effect on the clinical improvement of the stimulation caused by performing a cisternal puncture, one animal was injected with normal salt solution. This injection was without effect (dog No. 6, Table III). Wagging the tail and loss of apathy were taken as criteria of improvement. Such changes were but temporary, and in order to save the animal at the end of the experiment it was necessary to administer glucose intravenously.

Dog No. 5 was given a very large dose of insulin and severe shock with convulsions was produced. Repeated intraspinal injections of glucose solution failed to ameliorate the symptoms despite the fact that the spinal fluid sugar reached a level more than twice normal.

During the course of this work it was interesting to note the rapidity with which glucose, injected intravenously, penetrated into the spinal fluid.

Thus following the injection of 25 c.c. of 50 per cent glucose in seven minutes the spinal fluid sugar of dog No. 5 rose from a hypoglycemic value of 28 to 78 mg. per 100 c.c. Dog No. 6 showed a similar rise. However, such

TABLE III

THE EFFECT OF INTRASPINAL GLUCOSE INJECTIONS UPON INSULIN HYPOGLYCEMIA

| DOG NO.                                                                                                                                                | "SHOCK"<br>BLOOD<br>SUGAR<br>(MG. PER<br>100 C.C.) | "SHOCK"<br>SPINAL<br>FLUID<br>SUGAR<br>(MG. PER<br>100 C.C.) | INTRASPINAL<br>GLUCOSE                                                                                         | BLOOD<br>SUGAR<br>AFTER IN-<br>TRASPINAL<br>GLUCOSE<br>(MG. PER<br>100 C.C.) | RESULT                                                                                                                                                                                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------|--------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1<br>8 kg.<br>80 units<br>(subcuta-<br>neous)<br>11:30 A.M.                                                                                            | 1:40 P.M.<br>25                                    | 1:45 P.M.<br>24                                              | 3 c.c. of 2%<br>glucose<br>1:45 P.M.                                                                           | 32<br>1:55 P.M.                                                              | Dog lifted ears and began<br>to wag tail at once. After<br>5 min. seemed to be back<br>in shock. Was given 40<br>c.c. of 50% glucose in-<br>travenously. Dog died<br>during the night.                           |
| 6<br>10.3 kg.<br>60 units<br>(subcut.)<br>11:45 A.M.                                                                                                   | 1:15 P.M.<br>34                                    | 1:20 P.M.<br>28                                              | 2 c.c. normal<br>saline (intra-<br>spinally)<br>1:20 P.M.                                                      | 37<br>1:25 P.M.                                                              | No change noted. Given 25<br>c.c. of 50% glucose in-<br>travenously. Dog sur-<br>vived.                                                                                                                          |
| 2<br>11 kg.<br>110 units<br>(subcut.)<br>10:55 A.M.                                                                                                    | 12:55 P.M.<br>37                                   | 1 P.M.<br>23                                                 | 3 c.c. of 10%<br>glucose<br>1 P.M.                                                                             | 41<br>1:05 P.M.                                                              | Little, if any improvement.<br>Dog had to be given 40<br>c.c. of 50% glucose (in-<br>traven.) but showed poor<br>response. Died at 6 P.M.                                                                        |
| 7<br>10.2 kg.<br>80 units<br>(subcut.)<br>9:55 A.M.                                                                                                    | 11:55 A.M.<br>44                                   | 12 N.<br>33                                                  | 2.5 c.c. of 4%<br>glucose<br>12 N.                                                                             | 37<br>12:05 P.M.                                                             | Showed improvement for 15<br>min. but rapidly went<br>back into shock and was<br>given 25 c.c. glucose in-<br>travenously. Dog sur-<br>vived.                                                                    |
| 7<br>10.2 kg.<br>40 units<br>(intraspinal)<br>10:45 A.M.                                                                                               | 12:15 P.M.<br>33                                   | 12:20 P.M.<br>23                                             | 2 c.c. of 4%<br>glucose<br>12:20 P.M.                                                                          | 38<br>12:25 P.M.                                                             | Dog improved temporarily<br>but symptoms of shock<br>followed. Survived after<br>25 c.c. of 50% glucose was<br>given (intraven.).                                                                                |
| 8<br>12.6 kg.<br>9:55 A.M.<br>40 units<br>11:30 A.M.<br>80 units<br>12:35 P.M.<br>40 units<br>1:20 P.M.<br>40 units<br>(subcut.)<br>Total<br>200 units | 2:10 P.M.<br>43<br>3:40 P.M.<br>50                 | 2:15 P.M.<br>17<br>3:45 P.M.<br>160<br>4:40 P.M.<br>177      | 2:20 P.M.<br>2 c.c. 4%<br>glucose<br>3:50 P.M.<br>2 c.c. 4%<br>glucose<br>4:50 P.M.<br>2 c.c. of 4%<br>glucose | 3:40 P.M.<br>50                                                              | Dog showed no improvement<br>(permanent) in spite of<br>the fact that the spinal<br>fluid sugar was main-<br>tained at a high level.<br>Received no intravenous<br>glucose and died at 8<br>P.M. in convulsions. |

rapid penetration does not occur in nonhypoglycemic humans as was shown in the studies of Levinson.<sup>5</sup> Such rapid penetration of the meninges is probably not equaled by any other physiologic constituent.

## DISCUSSION

Cases of diabetes mellitus that present unmistakable symptoms of insulin shock when the blood sugar is at a relatively high level are not unusual. Such



TABLE IV  
SHOWING SPEED OF SPINAL FLUID PENETRATION FOLLOWING INTRAVENOUS GLUCOSE  
IN HYPOLYCEMIC DOGS

| DOG NO. | BLOOD SUGAR | SPINAL FLUID SUGAR |                                            |
|---------|-------------|--------------------|--------------------------------------------|
|         |             | BEFORE GLUCOSE     | AFTER 25 C.C. 50% GLUCOSE<br>INTRAVENOUSLY |
| 5       | 61          | 32                 | 78 (7 min. later)                          |
| 6       | 34          | 28                 | 73 (10 min. later)                         |

cases usually respond to carbohydrate ingestion, despite the fact that the blood sugar values may range between 150 and 200 mg. per 100 c.c., or more. John<sup>6</sup> has attempted to explain such cases of shock on the basis of a decreased permeability of the corpuscles in the diabetic patient. Other observers<sup>7, 8</sup> on the other hand have found an increased permeability of these cells. Our own work throws no additional light on this phase of the subject. It is apparent that meningeal permeability is quite high in the ordinary case of insulin hypoglycemia, and patients in such cases recover quite promptly following intravenous glucose injections; but it is conceivable that in such cases as the one reported here meningeal permeability may be markedly decreased. Under such circumstances insulin shock might persist even though, by intravenous glucose injections, high blood sugar values are obtained. Decreased meningeal permeability could be ascertained by spinal fluid sugar determinations and, if low values are found despite high blood sugar figures, intraspinal injections of glucose might be resorted to.

#### CONCLUSIONS

1. Insulin, in the normal animal, has a uniform action in lowering the spinal fluid sugar. Such decreases are often of greater proportion than the blood sugar reductions.

2. There is only a temporary improvement following intraspinal glucose injections in hypoglycemic dogs. A sustained high spinal fluid sugar without a commensurate high blood sugar will not preserve the life of the animal.

3. Glucose injected intravenously during hypoglycemia enters the spinal fluid very rapidly in the experimental animal.

4. Intraspinal glucose injections should be resorted to in the hypoglycemic diabetic who does not respond to intravenous glucose injections.

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# THE BLOOD LIPASE IN PATIENTS WITH PEPTIC ULCER\*

## ITS RELATION TO HEPATIC AND PANCREATIC DISEASE

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CHERRY and Crandall<sup>2</sup> found an enzyme capable of splitting olive oil in the blood of dogs after experimental damage to the pancreas. They<sup>2</sup> found the same lipolytic enzyme in 80 per cent of the cases of liver disease studied. In their opinion, the only conditions in which this enzyme appears in increased amount in the blood, both in the dog and in man, are pathologic changes in the liver and pancreas.

Occasional reference is made in the literature to lesions and dysfunction of the liver and pancreas associated with peptic ulcer. Thus, Gandy,<sup>3, 10</sup> in 1899 and again in 1920, mentioned lesions of the liver found in cases of peptic ulcer. Pathologic changes in the liver and kidneys have been suggested by LeNoir, Richet, and Jacquelin<sup>16</sup> as the cause of death of five patients following surgical procedures for peptic ulcer. Since the hepatic alterations could not be demonstrated by clinical methods they<sup>18</sup> employed laboratory tests. Unfortunately, some of the liver function tests they used were of questionable value, namely, provoked glycosuria and digestion hemoclasia. Because in a considerable percentage of cases of gastric ulcer the fasting blood sugar and the glycemic coefficient were greater than normal, LeNoir, de Fossey and Richet<sup>15</sup> concluded that the changes were due to disturbed hepatic function. That hepatorenal insufficiency may be found in cases of carcinoma of the stomach but is more frequent and more marked in peptic ulcer is the opinion of LeNoir, Richet and Jacquelin.<sup>17</sup> Vilardell and Lloret<sup>26</sup> demonstrated by means of biopsy definite microscopic lesions of the liver in patients with peptic ulcer.

Cases of clinically manifest jaundice associated with gastric and duodenal ulcer have been reported by Tiprez and Dumont,<sup>24, 25</sup> and the relation of jaundice to disease of the liver and biliary tract has been discussed by Surmont,<sup>23</sup> Bickel,<sup>1</sup> and Zoepffel.<sup>27</sup> Masked jaundice associated with peptic ulcer and demonstrated by means of the Van den Bergh reaction has been investigated by Hadlich,<sup>11</sup> and by Kalk and Siebert.<sup>14</sup> Cholecystitis and cholelithiasis have been found accompanying ulcer of the duodenum (McVicar and Weir,<sup>20</sup> Judd,<sup>13</sup> Bruce,<sup>2</sup> Mix,<sup>22</sup> MacLaren and Oertling<sup>19</sup>).

Meyer<sup>21</sup> reported two cases of renal glycosuria with ulcer of the duodenum and thought it might be due to an associated infection of the pancreas. Jankelson and Rudy<sup>12</sup> stated that a nondiabetic glycosuria in cases of peptic ulcer was suggestive of an adjacent chronic pancreatitis. The relationship between lesions of the pancreas and ulcers of the duodenum and stomach has been dis-

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cussed by Ehrström and Nylander.<sup>7</sup> Dysfunction of the pancreas has been investigated by means of spontaneous and provoked glycosuria, hyperglycemia, and the diastase content of the blood and urine by Galli, Pecco and Polacco.<sup>8</sup> These authors considered pancreatic dysfunction to have been present in 32 per cent of the cases studied.

In order to apply a more reliable test to the problem of the possible association of disturbance of the functions of the liver and pancreas with ulcer of the stomach and duodenum, we have employed the method described by Cherry and Crandall<sup>3</sup> and have studied the lipase content of the blood of fifty patients with peptic ulcer and of twenty-five control patients.

#### METHOD

In each of two test tubes 1 c.c. of blood serum was added to 3 c.c. of distilled water. One tube was inactivated at 90° C. for five minutes. This inactivated tube was used as the control. To each tube were added 2 c.c. of a 50 per cent emulsion of olive oil and 0.5 c.c. N/3 sodium phosphate buffer adjusted to  $P_H$  7.0. The contents of the tubes were thoroughly mixed and incubated at 40° C. for 24 hours. At the end of that time they were titrated with N/20 sodium hydroxide, using three drops of 1 per cent alcoholic solution of phenolphthalein as an indicator. The results have been expressed in cubic centimeters of N/20 alkali necessary to neutralize the fatty acids set free in 1 c.c. of olive oil.

In 60 determinations of inactivated serum it was found that the limits of variation were not more than 0.2 c.c. of N/20 NaOH. Since this corresponds with the results of Cherry and Crandall we have considered 0.2 c.c. as the limit of experimental error, and any reading of 0.3 c.c. or greater has been recorded as positive.

#### DISCUSSION

The results of the blood lipase determinations of fifty patients with peptic ulcer are found in Table I. In all cases except one roentgenologic evidences of peptic ulcer were present. The patient in case 8 was proved to have a duodenal ulcer at operation. A lipase titer of 0.3 c.c. or greater N/20 sodium hydroxide was found to be present in 38 cases, or 76 per cent. No relation between the lipase content of the blood and the age or sex of the patient could be demonstrated.

In most cases the clinical history was the only source of information available to us by which an estimate could be made as to the approximate duration of the ulcer. An appreciation of the questionable accuracy of this method was fully realized since it is well known that many cases of peptic ulcer come to necropsy although no definite symptoms of ulcer were present during life. However, in a few cases roentgenologic evidences and previous surgical operations gave a more accurate criterion as to duration. In our studies no connection could be found between the amount of blood lipase, as indicated by the alkali titer, and the approximate duration of the ulcer.

Only four cases of gastric ulcer were studied. The blood lipase titer of these patients was 0.5, 0.6, 0.6, and 0.4 c.c. N/20 sodium hydroxide. It seems probable, therefore, that the presence of an ulcer in either the stomach or duodenum may be associated with an increase of the blood lipase titer.

The effect of alimentary lipemia by increasing the total amount of fat for the enzyme to act upon, thus liberating more fatty acids, was considered. In the control cases no alteration in the titrations due to this factor was noted. The blood was taken from the ulcer patients before or after meals or after 12

TABLE I  
BLOOD LIPASE IN PEPTIC ULCER (TITER EXPRESSED IN C.C. OF N/20 NaOH)

| CASE | AGE | LOCATION | DURATION | LIPASE | CASE | AGE | LOCATION | DURATION | LIPASE |
|------|-----|----------|----------|--------|------|-----|----------|----------|--------|
| 1    | 55  | G        | 7y       | 0.5    | 26   | 55  | D        | 34y      | 0.4    |
| 2    | 44  | D        | 5m       | 0.4    | 27   | 50  | G        | 12y      | 0.6    |
| 3    | 25  | GJ       | ?        | 0.6    | 28   | 42  | D        | 9y       | 0.6    |
| 4    | 66  | D        | 2y       | 1.0    | 29   | 45  | D        | 17y      | 0.8    |
| 5    | 36  | D        | 12y      | 0.6    | 30   | 35  | G        | 5y       | 0.6    |
| 6    | 31  | D        | ?        | 0.1    | 31   | 20  | D        | 6m       | 0.8    |
| 7    | 48  | D        | 8y       | 0.8    | 32   | 26  | D        | 9y       | 0.2    |
| 8    | 34  | D        | ?        | 0.1    | 33   | 45  | D        | 12y      | 1.5    |
| 9    | 49  | D        | ?        | 0.2    | 34   | 60  | G        | 15y      | 0.4    |
| 10   | 45  | D        | ?        | 1.0    | 35   | 33  | D        | 9y       | 0.5    |
| 11   | 59  | D        | 2m       | 0.6    | 36   | 53  | D        | 10m      | 0.4    |
| 12   | 25  | D        | 2m       | 0.3    | 37   | 53  | D        | 6y       | 0.1    |
| 13   | 24  | D        | 1m       | 1.4    | 38   | 51  | D        | 4y       | 0.4    |
| 14   | 38  | D        | 2y       | 0.3    | 39   | 38  | D        | 4y       | 0.4    |
| 15   | 68  | D        | 6y       | 0.3    | 40   | 34  | D        | 10m      | 0.4    |
| 16   | 41  | D        | 21y      | 0.6    | 41   | 47  | D        | 2y       | 0.5    |
| 17   | 44  | D        | 1y       | 0.3    | 42   | 40  | D        | 6y       | 0.6    |
| 18   | 37  | D        | 10y      | 0.5    | 43   | 53  | D        | 3y       | 0.2    |
| 19   | 38  | D        | 3y       | 0.6    | 44   | 46  | D        | 8y       | 0.4    |
| 20   | 26  | D        | 5y       | 0.3    | 45   | 44  | D        | 10y      | 0.9    |
| 21   | 51  | D        | 3y       | 0.1    | 46   | 30  | D        | 6y       | 0.2    |
| 22   | 33  | D        | 7y       | 0.2    | 47   | 26  | D        | 8m       | 0.4    |
| 23   | 45  | D        | 18y      | 0.5    | 48   | 36  | D        | 3y       | 0.4    |
| 24   | 36  | D        | 14y      | 0.0    | 49   | 42  | D        | 8m       | 0.7    |
| 25   | 42  | D        | 6m       | 0.2    | 50   | 61  | D        | 6m       | 0.0    |

All were males except 21, 34, 49, and 50.

The Wassermann reaction was positive in 7 and 13 and negative or undetermined in the remainder.

Nine patients had some type of surgical procedure on the stomach, usually a gastroenterostomy.

Various types of therapy were used: alkalis, citrosalvan, mucin, or chondroitin.

hours of fasting. Alimentary lipemia either caused no change in the lipase titer, or it was so small as to be negligible.

Crandall and Cherry<sup>4</sup> found in 146 control patients that six gave a titration of 0.3 c.c. or more N/20 NaOH. In order to check our technic twenty-five unselected patients were studied. After making the determinations, none of the patients were found to have symptoms referable to hepatic disease or peptic ulcer. One patient was said to have "some pancreatic involvement." Three patients in this group gave positive results. The lipase titer and diagnosis in these cases were as follows: 0.5 c.c. N/20 sodium hydroxide, hypertension, nephrosclerosis and arteriosclerosis; 0.5 c.c. N/20 sodium hydroxide, possible kinking of the right ureter; 1.2 c.c. N/20 sodium hydroxide, diabetes mellitus, pyelitis, cystitis, and nephritis.

Evidence has recently been presented by Ivy, Schrager, and Morgan<sup>28</sup> that disturbance of hepatic function may be an etiologic factor in the occurrence of gastric and duodenal ulcer. The experiments here reported throw no light on this problem. Although we found positive evidence of disturbance of the

function of the liver or pancreas or of both together in 76 per cent of the cases of gastric and duodenal ulcer studied by a new and apparently reliable test, the lesions underlying these functional disturbances might just as well have been secondary to the ulcer as a primary and etiologic factor in its production.

### CONCLUSIONS

1. An enzyme capable of splitting olive oil was found to be present in the blood of 38 (76 per cent) out of 50 patients with peptic ulcer.
2. The amount of this enzyme present, indicated by its alkali titer, had no relation to the probable duration of the ulcer or to the age or sex of the patient.
3. It is suggested that this lipolytic enzyme in the blood is due to associated dysfunction of the pancreas or liver or both.

4. This test may prove valuable in preoperative determination of the presence of one factor of the surgical risk, namely, pathologic changes in the structure or function of the liver and pancreas, in patients with peptic ulcer.

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## CARCINOMA OF THE PANCREAS\*

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IN AN attempt to determine the frequency and symptomatology of carcinoma of the pancreas we have reviewed 50 cases proved at autopsy, seen during the period from January 1, 1925, to January 1, 1933. The records are from the Cook County Hospital, and a smaller number are from the Research and Educational Hospital. The majority of the autopsies were performed by Dr. R. H. Jaffé, pathologist at the Cook County Hospital. Clinically diagnosed cases, even those confirmed by laparotomy but not proved by necropsy, are omitted because of the great difficulties in diagnosis. Metastatic carcinoma of the pancreas is not mentioned.

Knowledge of carcinoma of the pancreas certainly goes back to 1857, when the elder Da Costa<sup>1</sup> wrote of it. Mondiere<sup>2</sup> in 1836 is quoted as having written about it. Ancelet<sup>3</sup> in 1864 includes mention of it in his monograph. However Bard and Pic<sup>4</sup> in 1888 first described the clinical picture accepted as classical of carcinoma of the head of the gland. Knowledge of this last work has gradually pervaded the whole of medical literature. French writers since that time have accorded carcinoma of the pancreas a relatively important place in their medical literature.

The 50 cases occurring in 7,932 autopsies constitutes 0.63 per cent of all necropsies. In this group there were 942 malignancies of all sorts. Primary carcinoma of the pancreas made up 5.3 per cent of all malignancies. Carcinoma of the stomach was seen 211 times, 22.3 per cent of all malignancies. The ratio between the number of carcinomas of the stomach to those of the pancreas is roughly 4 to 1. Kolb,<sup>5</sup> in 1907, found 54 carcinomas of the pancreas in men among 8,777 deaths due to malignancy, and 50 in 11,266 similar cases in women. There was in his statistics a ratio of 87 carcinomas of the stomach to one carcinoma of the pancreas in male. Our figures are more in agreement

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with figures from the Massachusetts General Hospital.<sup>6</sup> Here in 700 necropsies, 14 carcinomas of the pancreas were found while there were only 17 cancers of the stomach. Ewing<sup>7</sup> from large statistics states that carcinoma of the pancreas constitutes from 1 to 2 per cent of all malignancies. Of Kauffman's<sup>8</sup> cases of malignancy examined postmortem, 1.76 per cent were in the pancreas.

In our series of cases males were much in the majority, as 41 were in men and only 9 in women. Correcting for the preponderance of postmortems on men, the authors find the incidence would be 41 men to 18 women. In most series described in the literature this ratio holds.

|                       | TOTAL CASES | MEN | WOMEN | HEAD | BODY AND<br>TAIL | ENTIRE<br>GLAND |
|-----------------------|-------------|-----|-------|------|------------------|-----------------|
| Mirallie <sup>9</sup> | 104         | 69  | 35    | 39   | 7                | 19              |
| Speed <sup>10</sup>   | 52          | 36  | 16    |      |                  |                 |
| Fuchter <sup>11</sup> | 31          | 22  | 9     |      |                  |                 |
| Kiefer <sup>12</sup>  | 33          | 18  | 15    | 24   | 5                | 4               |
| Wallau <sup>13</sup>  | 330         | 196 | 134   | 169  | 46               | 125             |
| Ewing                 | 354         |     |       | 156  | 40               | 158             |
| Oser <sup>14</sup>    | 26          |     |       | 20   | 5                | 1               |

Though a fair share (38.4 per cent) of the autopsies are on colored people, only five of our patients were negroes.

Of the 50 in this series, in only 22 was the growth limited to the head of the gland. In 23 cases the neoplasm arose in either the body or tail. In only four cases did the neoplasm occupy the entire pancreas. One was from an aberrant pancreas in the wall of the duodenum. The high proportion of carcinoma of the body and tail, namely 46 per cent, is in striking contrast to the figures of others.

All cases in which the carcinoma arose in the common duct have been excluded. As pointed out by Shapiro and Lifvendahl,<sup>15</sup> who worked with practically the same material, neoplasms of the biliary passages are often mistaken for carcinoma of the head of the pancreas even at laparotomy. They showed that biliary duct carcinoma occurred much more frequently than carcinoma of the head of the pancreas.

#### ANATOMIC FINDINGS

Grossly the tumors of the pancreas ranged from 3 cm. in greatest diameter to as large as 12 cm. The consistency ranged from firmness to stony hardness. Characteristically their growth was invasive into the neighboring organs and the remainder of the pancreas. In no case was there any semblance of a capsule. White streaks often outlined the lymphatics through which the tumor had extended.

Microscopically it was found that 27 of the tumors were the adenocarcinoma form; 16 were scirrhus; two, medullary; two, alveolar; and one, simplex. Two are unclassified types of highly undifferentiated carcinoma. The microscopic diagnosis bore no relation to the duration of the disease or the extent of metastases. The three cases in which there were no metastases were all scirrhus.

The pancreatic ducts were often obstructed and hence distended in the distal part of the gland. The circumference of the dilated duct commonly reached 30 mm. The gallbladder was found dilated in all but two of the 19 cases of carcinoma of the head of the pancreas with jaundice, and one of these patients had had a cholecystectomy years before. Courvoisier's rule holds, therefore, in roughly 95 per cent of these cases. However in only 6 patients were the clinicians able to palpate the gallbladder.

Metastases of these tumors are very important since they dominate the clinical and pathologic picture in many patients. In only three patients were there no metastases; all three tumors were in the head of the gland and were of the scirrhus type. The lymph nodes in the upper abdomen and the liver were most regularly affected, each showing metastases in 68 per cent of the cases. Carcinomatosis peritonei was found in 22 cases, though only 9 had an ascites that was demonstrable clinically. Lymphatic spread to the mediastinal glands and the pleura was quite frequent. Even a carcinomatous lymphangitis of the right upper lobe was found in one case. Liver metastases, contrary to being small as described by Bard and Pic are of fair size averaging from 3 to 7 cm. and reaching 15 cm. in diameter.

Involvement of the stomach by direct extension of the neoplasm occurred six times, all in carcinomas of the body or tail of the gland. The duodenum was invaded seven times. This anatomic lesion will explain the common finding of blood in the stools, as well as the frequent false results of roentgenologic examination of the stomach.

Bizarre metastases are quite frequent and often occur early in the course of this disease. One carcinoma had invaded both ureters from retroperitoneal metastases and given rise to hematuria. Another had extensively riddled the submucosa of the entire small bowel. One invaded the transverse colon causing an obstruction. A fourth had caused a kink at the splenic flexure by fixing its supporting mesocolon with carcinomatous infiltration. Only one had metastases at the umbilicus. Only one had invaded the bones, the invasion being the result of direct extension from metastases in the mediastinal lymph nodes.

In contrast to the lymphatic spread which was so common, distant hematogenous metastases<sup>16</sup> were found in only two cases. Both showed lymphatic spread from a primary tumor of the body of the gland as well. The first such case had an extensive lymphatic spread to all the viscera of both chest and abdomen, and in addition had multiple intracutaneous nodules of carcinoma over the face, neck, and shoulders. Another case showed involvement of the retroperitoneal lymph nodes and also had an isolated metastasis in the myocardium.

#### PATHOGENESIS

There is no apparent connection between carcinoma of the pancreas and regeneration. Cirrhosis of the liver with associated cirrhosis of the pancreas is rarely followed by carcinoma of the pancreas. Only three such cases were found in the literature.<sup>17</sup> In these cases proliferation of the duct epithelium may be a precancerous lesion. In cases of chronic obstruction to the pan-



creatic ducts from any cause, the acini may atrophy while the duct system often proliferates producing buds of tissue like the islands of Langerhans. One of this series showed a moderate degree of this process. In three cases, multiple small congenital cysts were found in the pancreas, whose occurrence might suggest the theory of carcinoma arising from misplaced embryonic tissue, the cell rests of Cohnheim or the hamartomas of Albrecht. Interstitial pancreatitis as a precursor to carcinoma was not found here. Gallstones were an associated observation in only 6 of the 50 cases.

T. Cherry<sup>18</sup> of Melbourne produced carcinoma of the pancreas in mice, experimentally, by inducing chronic inflammation of the area by the injection of tubercle bacilli to which mice are resistant. He showed carcinoma arising from the ducts in these animals, with intermediate stages present.

Among these 7,932 necropsies, there were found two small adenomas of the islands of Langerhans. One was seen in a pancreas in which a carcinoma had arisen from the ducts. The other adenoma was purely an incidental finding at autopsy. Neither of these patients presented any symptoms of hyperinsulinism.

#### CLINICAL RECORDS

Since three patients entered the hospital moribund, and no adequate history could be obtained from another patient, the study is limited to 46 clinical records. Of these cases the duration of the process judged from the onset of the first symptom that could be ascribed to the lesion in question, varied from two weeks to three years for an average of 6.9 months. Only five said their trouble was of more than one year's standing.

Pain was the first symptom noted in 23 cases. Weight loss was noted first in 6, jaundice in 6, anorexia in 7, abdominal distension in 3, and edema of the legs in one. Pain and weight loss were the most constant symptoms occurring in 43 cases, or 93.4 per cent; next was anorexia in 30 cases or 65 per cent; then jaundice in 23 cases or 50 per cent; constipation in 17, or 36.9 per cent; vomiting in 16, or 34.8 per cent; and nausea alone in ten others. Diabetes was present in eight, or 17.4 per cent. Polyphagia was present in three cases. Diarrhea was a complaint of only one patient. Kiefer in his series of 33 found cachexia, including loss of weight to be the most common symptom, jaundice second, and pain third. Speed in his group also found these three symptoms to be the most common in the order named.

Pain was undoubtedly the most constant symptom. Kiefer reports it as a symptom in 21 of 33 patients. Speed has it recorded in 61 per cent of 52 cases, while Mirallie writes of it as an important symptom in 56 of 113 patients. The pain was most frequently epigastric in location, being there in roughly three-fourths of all the patients. It varied from a soreness to a most excruciating type of paroxysm requiring narcotics for relief. It occasionally radiated around the costal margins and in a few instances even up into the chest. In the case with ureteral involvement, pain radiated down to the groins. Little relation was noted between the pain and the time or type of food-taking; however, it was frequently produced at the onset by taking very large meals and fatty foods. In only two instances it was partially relieved by alkalis or by

vomiting. These two patients got relief with Sippy ulcer management for a period of two weeks, after which that was not effective.

Pain in the back occurred in association with epigastric pain in 15 cases while it was limited to the back in 2 patients. This symptom, pain in the back, has been noted in the literature particularly by Chauffard,<sup>19</sup> who in 1908 drew attention to its presence in carcinoma of the body of the pancreas. The pain is characteristically located in the lower dorsal and upper lumbar regions of the back, and is most often to the left of the midline. It is severe and relieved in many instances only by the *upright position of the body* as by sitting up, walking with the body bent forward at the hips, or lying curled up on the right side. Lying on the back seemed to aggravate the pain, and so quite often the patients described the pain as being worse at night or said that it first bothered them during the night. Of the 15 cases in which the pain occurred in the back, the tumor was found in 9 to involve the body or tail of the gland; while 4 were primarily in the head, and two occupied the entire organ. Eleven of the 15 patients with pain in the back showed at autopsy a carcinomatous involvement of the body of the pancreas, which suggests that this symptom may be of diagnostic significance in doubtful cases. As to the way the pain is produced, obstruction of the pancreatic duct with its subsequent distension is one theory; just as dilatation of the biliary ducts is thought to cause pain. Invasion of the celiac plexus by the newgrowth is the most commonly accepted explanation. Many cancers which have retroperitoneal metastases, as carcinoma of the stomach, liver, or lung, produce pain in the back as a distressing symptom, even in the absence of metastases to the spine. We have not observed any postural relief of the pain in any of these other tumors.

Anorexia was a major complaint of 21 cases being associated slightly more often with carcinoma of the head (56 per cent) than with carcinoma of the body of the gland (47.5 per cent). Nausea and vomiting usually followed in sequence. The loss of appetite bore no relation to the presence of icterus or to duodenal obstruction. In fact of 13 showing direct invasion of the duodenum or stomach, only 5 patients had anorexia among their chief entering complaints.

Hematemesis did not occur in any of this series even in those with malignant ulcers in the stomach although 8 of the 21 cases having gastric analyses showed occult blood in the stomach content.

Jaundice, so strikingly a part of the picture of carcinoma of the pancreas since Bard and Pic wrote of it, was by no means constantly present. It occurs here in 23 of the 50 cases. It was the initial symptom in only 6 cases. It was absent in 3 of the 22 cases having carcinoma of the head of the pancreas. Since the jaundice is due to the compression of the common duct by tumor tissue, no jaundice follows when the tumor spreads in such fashion as to miss the common duct. Jaundice was present in 3 of the 4 cases of carcinoma of the entire gland, and appeared once as a result of compression of the hepatic ducts by a metastasis from a cancer in the body of the gland. Once the jaundice appeared it did not recede, save in one woman who was icteric for 4 months and recovered only to have a recurrence of the icterus 6 months later, this time with weight loss and a progressive downhill course. The icterus indices ranged up to 250.

Jaundice and pain appeared often together for of 19 cases with obstruction of the common duct due to malignancy, there was severe pain in 17 instances. Though usually in the right upper quadrant the pain varied somewhat. E.N. had his pain in the back on the left side all the time. The pain in J.B. was of ulcer type and responded to Sippy management for a time. L.S. had only a slight epigastric distress though there was a carcinoma completely obstructing the common duct and causing a very large gallbladder. J.C. alone of this icteric group was entirely free from pain. In general when present with jaundice in cancer of the head of the pancreas, the pain was usually moderately severe, aching in character, and spontaneously remittent for short periods. It bore no definite relation to postural relief save in one case. At no time had there been colics requiring morphine.

Weight loss ranged from 10 to 80 pounds, averaging 37 pounds. It was the first symptom in 7 patients and had taken place in from two weeks to one year. Kiefer described an average weight loss of 28 pounds and stressed the speed with which it took place.

Diabetes was present in eight cases, or 17.8 per cent. This is a much higher proportion of diabetes than others have given. Joslin<sup>20</sup> says the association is very unusual and quotes from Kiefer. Three of the patients had known of their diabetes before they entered the hospital. One of the latter first knew of her glycosuria when she consulted a physician because of epigastric pain and pruritus vulva. The blood sugar values on these 8 patients ranged from 166 to 250 mg. per cent. In 2 of these 8 cases the entire gland was affected while in 3 the tumor was in the body, and the remaining three were primarily in the head of the pancreas. In the latter three one had an atrophy of the distal portion of the gland, and another showed multiple cysts occupying the body and tail of the gland with a virtual destruction of the parenchyma.

Polyphagia was present in three cases. Two of these had diabetes to which the symptom may be ascribed. The remaining case showed no other manifestation of a suspected hypoglycemia and did not have an adenoma of the island tissue at necropsy, but instead a scirrhous carcinoma. Not a single case presented the symptom complex described by Wilder et al.<sup>21</sup>

Diarrhea was present in only 1 of the 46 patients. Constipation was a complaint that had been present in 17 patients for variable periods up to many years. Without a single case of fatty stools recorded this lone instance of diarrhea would indicate that loose fatty stools with jaundice occur less frequently than is supposed. The frequency of constipation only emphasizes the rarity of diarrhea.

#### PHYSICAL FINDINGS

On examination of the abdomen a tumor mass was palpable in 35 cases. In 25 these masses were in the right upper quadrant and had been called metastatic tumors of the liver. In 7 instances the mass was limited to the epigastrium or spread to the left hypochondrium. In 5 cases both upper quadrants showed masses. All of the masses were hard, only a few nodular. As a rule the masses in the epigastrium were fixed, but in at least two instances in

which the carcinoma protruded anteriorly from the body of the gland, a movement of 3 cm. or more with each respiration was noted.

Ascites was found in 8 cases on clinical examination. Each was due to a carcinomatosis peritonei. In one instance the carcinoma nodules could be palpated through the abdominal wall. Rectal examination in three cases revealed hard lumps in the culdesac which proved to be drop metastases. Two had been thought to be a primary carcinoma of the rectum.

Fever was present during the hospital stay of 10 patients, or 21 per cent. The temperatures ranged from 97° to 102° F. Bard and Pic thought these cases all had subnormal temperature.

#### ROENTGEN FINDINGS

The x-ray examination was found to be of little help in the diagnosis in most instances. On the contrary misleading information was not infrequently obtained. Of 6 cases in which fluoroscopic examinations revealed stomach defects, 4 were infiltrations of the greater curvature by the pancreatic newgrowth. Two more were reported as pyloric obstruction. In a patient having metastases to the mediastinal glands the roentgenogram was that of a carcinoma of the esophagus. A single case showed what was taken to be an extragastric mass with displacement of the stomach. One that had invaded the transverse colon gave the findings of a carcinoma of the transverse colon with obstruction. Scholz and Pfeiffer<sup>22</sup> reported two cases of carcinoma of the tail of the pancreas showing a deformity of the greater curvature of the stomach due to an extragastric mass. On its presence one of their cases was accurately diagnosed. In carcinoma of the head of the gland a widening and stasis of the C-shaped duodenal loop has been described but was seen here only in the large tumor arising from an aberrant pancreas in the wall of the third portion of the duodenum. X-ray examination is chiefly helpful in these cases by ruling out pathologic processes in the stomach or duodenum.

Upon review of these clinical manifestations of carcinoma of the pancreas as shown by our series of proved cases, one is unable to point out anything absolutely characteristic. On the contrary the difficulties of an accurate presumptive diagnosis is impressed upon us. Carcinoma of the head of the gland affords perhaps the best opportunity of diagnosis with the symptoms of pain, icterus, and weight loss pointing the way. The jaundice is not, however, painless as has been stated, for pain was present in 17 of the 19 of this type of patient with icterus. Pain must be taken as a cardinal sign. We present the following as a typical case history of a patient with a carcinoma of the head of the gland.

#### CASE REPORTS

B. S., a white male, fifty-four years old, entered the Cook County Hospital July 8, 1925, and remained until September 3, 1925. He said he had been well except for a gradual loss of weight of 20 pounds in the preceding six weeks. Two weeks before entrance he had noted jaundice and marked weakness. Anorexia had appeared at the same time. During the two weeks before entrance he had taken only a little tea and cake. Pain in the right upper quadrant had been present only one week. It was dull and aching and though bothersome did not require narcotics. It did not radiate. He said that it went away if he lay quiet. He had

noticed white stools for the past two weeks. During the same time his urine was very dark. He did not have a diarrhea. Physical examination revealed a white laborer who had lost some weight but was not acutely ill. He was afebrile. The abdomen was rounded, soft and tympanitic, the liver firm and enlarged. Its edge was felt 7 cm. below the right costal arch in the nipple line. No nodules were made out in it. The rectal examination did not detect any masses. At exploration there was found ascites. The liver presented multiple metastatic nodules. There was a hard nodular mass in the region of the head of the pancreas. The gall-bladder was grossly distended. The patient died in less than three months still very deeply jaundiced, and terribly emaciated. His pain had persisted throughout the course of his illness. At autopsy a carcinoma of the head of the gland was found, with complete obstruction to the pancreatic and common bile ducts. Small metastases were present in great numbers of the liver. There were small metastases in the retroperitoneal lymph nodes. The primary tumor had invaded the second portion of the duodenum and ulcerated through the mucosa.

When the tumor involves the body and tail of the gland the diagnosis is even more uncertain but in certain cases, can be presumed. In a given case where a malignancy is suspected because of the age of the patient, loss of weight, epigastric distress, and especially pain in the upper lumbar area which responds to no simple measures save perhaps a change of posture, and with no other definite source of the tumor demonstrated, even by x-ray examination, carcinoma of the body or the tail of the pancreas is to be strongly considered. In those patients having diabetes, the attention will be focused on the pancreas. Where there has been an extension of the process to the stomach, the entire picture may exactly simulate carcinoma of the stomach.

The following case is presented as typical of carcinoma of the body of the pancreas:

I. B., a colored woman, aged sixty-one years, service of Dr. E. F. Foley, entered Cook County Hospital on June 9, 1931, and remained for seventy-five days. Her entrance complaint was of an epigastric pain, which had been present for five months, and a pain in the back, which had been present for three months. She had lost about 50 pounds in weight in the preceding six months. The epigastric pain was most intense slightly to the left of the midline. It reached its height about two hours after meals, but was present all the time. She described it as an aching or gnawing pain. Neither soda nor food brought relief. Though she was not hungry and occasionally was nauseated, she did not vomit and had not induced vomiting. The pain in the back appeared two months after the onset of the epigastric pain, which had gradually spread around either costal arch to the back. The upper lumbar and lower thoracic areas were affected, particularly on the left side. This pain was at its worst when she lay on her back. Sitting up and bending forward diminished the pain. She bent forward on walking, but could stand erect for a few minutes at a time.

Physical examination showed a colored woman who had lost much flesh, but still weighed 160 pounds. Her temperature was 99.6° F. and ranged down to 97° F. in the mornings. She was not icteric. All the physical findings were in the abdomen which was rounded and soft. There was a poorly outlined hard mass in the right upper quadrant. The kidneys and spleen were not felt. An ascites was demonstrated by the presence of shifting dullness. Rectal examination showed a hard mass about walnut size fixed in the culdesac.

Her clinical course was progressively downhill. The pain and weight loss persisted. An aversion to food developed six weeks after entrance. The ascites became so marked that she was twice tapped with removal of 2,500 c.c. and again 2,000 c.c. of a clear yellow fluid with a specific gravity of 1.020. No blood was present in this fluid. The result of the laboratory study was as follows: The urine was persistently negative for albumin, sugar, or bile. The stools were negative for blood at all times. The stomach contents showed an average free acidity of seven degrees, and a total acidity of ten. The hemoglobin was 60,

the white blood count averaged 10,300, and the red count stayed close to the entrance count of 3,700,000. The blood sugar was 85 mg. per 100 c.c.; the icterus index was 4. The x-ray examination showed nonvisualization of the gallbladder after oral administration of the dye. No anomaly was found in the stomach or duodenum. On two occasions a constant deformity with constriction of the hepatic flexure of the colon was demonstrated with barium enema.

At autopsy a carcinoma measuring  $8 \times 5 \times 4$  cm. was found in the body of the pancreas. There was an ascites due to carcinomatosis of the peritoneum. Numerous metastases were present in the liver. The hepatic flexure of the colon was bound to the undersurface of the liver by old adhesions.

#### COMMENT

It is obvious that because of the difficulties of diagnosis and the short average duration, the treatment is only palliative. The difficulties offered to surgery by the anatomic position of the pancreas and its close relation to the celiac plexus make extirpation almost hopeless, even if a diagnosis were made. As a palliative measure cholecystenterostomy has been done chiefly for relief of the icterus. A single case in this group had survived a cholecystogastrostomy. This patient was relieved of his jaundice for four months when it recurred and persisted until his death eight months postoperatively. His pain was not relieved though Speed says much relief is frequent. There is no reason to think a simple exploratory laparotomy will relieve the pain.<sup>23</sup>

#### SUMMARY

1. Primary carcinoma of the pancreas comprises 5.3 per cent of all carcinomas. It occurs about one-fourth as frequently as carcinoma of the stomach.

2. Carcinoma of the body and tail is as common as carcinoma of the head of the gland.

3. The most frequent symptoms of carcinoma of the pancreas are pain, weight loss, anorexia, jaundice, and nausea with vomiting. Diarrhea is quite rare.

4. Carcinoma of the head of the pancreas produces in three-fourths of the cases a progressively intense, moderately painful jaundice due to obstruction of the common bile duct. This cannot be differentiated from carcinoma of the biliary passages, a much more common lesion.

5. Carcinoma of the body or tail of the pancreas may be a presumptive diagnosis in the patient who is suspected of having an intraabdominal malignancy and who presents boring epigastric and upper lumbar pain partly relieved by postural changes, provided that no other primary tumor can be demonstrated. The presence of diabetes further supports this diagnosis.

6. The early occurrence of widespread metastases and the rapid progress of the disease make the treatment purely palliative.

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## THE USE OF SYNTHALIN IN DIABETES MELLITUS\*

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THE purpose of this experiment was to study the action of synthalin† on the glucose metabolism in a case of diabetes mellitus.

The product used was decamethylin-diguanidin, named synthalin by Frank, Nothmann and Wagner<sup>1, 2</sup> in Breslau and first used by them in the treatment of diabetes mellitus. It was reported to have an insulin-like action when given by mouth.

### CASE REPORT

T. Q., male, aged forty-six, weighed 69 kg. This patient first developed symptoms of diabetes mellitus at the age of forty-three during an attack of pneumonia, which was followed by empyema; and at the time of the experiment he had been diabetic for three years. The diabetes had only been accurately controlled for the three weeks prior to this experiment. With a diet of carbohydrate, 76 gm.; protein, 57 gm.; fat, 183 gm.; or available glucose, "G,"† 127 gm.; cal., 2,179, and insulin, 20 units before breakfast and 10 units before the evening meal, the urine was free of sugar. He was an intelligent and well-trained diabetic patient.

### EXPERIMENT I

*Method of Study.*—The diet was made up of 800 gm. of whole milk and 800 gm. of 20 per cent cream. One third of this mixture was given between

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†The preparation used in this experiment was obtained from C. F. Kahlbaum through Eli Lilly & Co., of Indianapolis, Indiana.

‡"G," or available glucose, was calculated by the formula, "G" = C + 0.58P + 0.10F.

TABLE I  
FIRST EXPERIMENT

| DATE     | WEIGHT<br>IN KG. | DIET                               | G   | FA<br>G | VOLUME OF<br>URINE | SPECIFIC<br>GRAVITY | GRAMS OF<br>GLUCOSE IN<br>URINE BY<br>FOLIN-<br>BERGUND | FASTING<br>BLOOD SUGAR<br>CONCENTRATION<br>IN PER CENT | GRAMS OF<br>NITROGEN<br>IN URINE | GRAMS OF<br>CREATININE<br>IN URINE | REMARKS                                                  |
|----------|------------------|------------------------------------|-----|---------|--------------------|---------------------|---------------------------------------------------------|--------------------------------------------------------|----------------------------------|------------------------------------|----------------------------------------------------------|
| April 22 | 71               | C-80<br>P-48<br>F-192<br>Cal.-2240 | 127 | 1.5     | 730                | 1.014               | 0.26                                                    |                                                        |                                  |                                    | Insulin 20-0-10                                          |
| April 23 | "                | "                                  | "   | "       | 1170               | 1.010               | 0.77                                                    | 0.197                                                  | 7.8                              |                                    | Insulin 20-0-10                                          |
| 24       | "                | "                                  | "   | "       | 1790               | 1.012               | 19.07                                                   | 0.202                                                  | 7.2                              |                                    | Insulin stopped                                          |
| 25       | "                | "                                  | "   | "       | 1400               | 1.014               | 24.18                                                   | 0.182                                                  | 6.9                              |                                    | Insulin 0-0-8                                            |
| 26       | "                | "                                  | "   | "       | 790                | 1.025               | 17.95                                                   | 0.180                                                  | 10.3                             |                                    | Insulin 8-0-0                                            |
| 27       | "                | "                                  | "   | "       | 1000               | 1.016               | 8.60                                                    | 0.188                                                  | 8.1                              |                                    | Insulin 8-0-0                                            |
| 28       | "                | "                                  | "   | "       | 1080               | 1.017               | 11.68                                                   |                                                        |                                  |                                    | B. M. R. = 0%                                            |
| 29       | "                | "                                  | "   | "       | 1060               | 1.015               | 7.27                                                    | 0.201                                                  | 7.3                              |                                    | Insulin 8-0-0                                            |
| 30       | "                | "                                  | "   | "       | 1320               | 1.010               | 5.28                                                    | 0.192                                                  | 7.3                              |                                    | B. M. R. = -1%                                           |
| May 1    | "                | "                                  | "   | "       | 1540               | 1.012               | 5.77                                                    | 0.191                                                  | 8.3                              |                                    | Insulin 8-0-0                                            |
| 2        | "                | "                                  | "   | "       | 1260               | 1.012               | 6.71                                                    | 0.205                                                  | 9.7                              |                                    | B. M. R. = -2%                                           |
| 3        | "                | "                                  | "   | "       | 690                | 1.016               | 3.77                                                    | 0.174                                                  | 6.1                              |                                    | Insulin 8-0-0                                            |
| 4        | "                | "                                  | "   | "       | 1060               | 1.010               | 4.24                                                    | 0.190                                                  | 7.8                              |                                    | Insulin 8-0-0 - - -<br>Synthalin 0.0125 gm.<br>at 8 A.M. |



TABLE I—CONT'D

| DATE  | WEIGHT<br>IN KG. | DIET | G | FA<br>G | VOLUME OF<br>URINE | SPECIFIC<br>GRAVITY | GRAMS OF<br>GLUCOSE IN<br>URINE BY<br>FOLIN-<br>BERGAND | FASTING<br>BLOOD SUGAR<br>CONCENTRATION<br>IN PER CENT | GRAMS OF<br>NITROGEN<br>IN URINE | GRAMS OF<br>CREATININE<br>IN URINE | REMARKS                                                  |
|-------|------------------|------|---|---------|--------------------|---------------------|---------------------------------------------------------|--------------------------------------------------------|----------------------------------|------------------------------------|----------------------------------------------------------|
| May 5 | "                | "    | " | "       | 1240               | 1.012               | 1.28                                                    | 0.163                                                  | 6.9                              |                                    | Insulin 8-0-0 -- --<br>Synthalin 0.0125 gm.<br>at 8 A.M. |
| 6     | "                | "    | " | "       | 1300               | 1.010               | 1.49                                                    | 0.141                                                  | 9.0                              |                                    | Insulin 8-0-0 Synthalin<br>0.025 gm. at 8 A.M.           |
| 7     | "                | "    | " | "       | 1340               | 1.007               | 0.52                                                    | 0.133                                                  | 7.6                              |                                    | Insulin 8-0-0 Synthalin<br>0.025 gm. at 8 A.M.           |
| 8     | "                | "    | " | "       | 1220               | 1.009               | 0.61                                                    | 0.148                                                  | 7.4                              |                                    | Insulin 8-0-0 Synthalin<br>0.025 gm. at 8 A.M.           |
| 9     | "                | "    | " | "       | 1080               | 1.010               | 1.24                                                    | 0.125                                                  | 7.8                              |                                    | Insulin 8-0-0 Synthalin<br>0.025 gm. at 8 A.M.           |
| 10    | "                | "    | " | "       | 900                | 1.013               | 0.88                                                    | 0.095                                                  | 6.9                              | .900                               | Insulin 8-0-0<br>Synthalin stopped                       |
| 11    | "                | "    | " | "       | 2050               | 1.008               | 2.05                                                    | 0.161                                                  | 8.7                              | 1.102                              | Insulin 8-0-0                                            |
| 12    | "                | "    | " | "       | 1700               | 1.006               | 1.37                                                    | 0.130                                                  | 8.4                              | 1.259                              | Insulin 8-0-0                                            |
| 13    | "                | "    | " | "       | 1080               | 1.014               | 2.82                                                    | 0.135                                                  | 6.1                              | 1.059                              | Insulin 8-0-0                                            |
| 14    | "                | "    | " | "       | 1360               | 1.012               | 2.86                                                    | 0.148                                                  | 7.6                              | 1.183                              | Insulin 8-0-0                                            |
| 15    | "                | "    | " | "       | 1290               | 1.011               | 1.09                                                    | 0.154                                                  | 8.1                              | 1.240                              | Insulin 8-0-0                                            |
| 16    | "                | "    | " | "       | 1400               | 1.012               | 2.24                                                    | 0.145                                                  | 8.4                              | 1.077                              | Insulin 8-0-0                                            |
| 17    | "                | "    | " | "       | 1210               | 1.016               | 1.69                                                    | 0.167                                                  | 6.2                              | 0.864                              | Insulin 8-0-0                                            |

TABLE II  
SECOND EXPERIMENT

| DATE   | WEIGHT<br>IN KG. | DIET                               | OR AVAIL-<br>ABLE GLU-<br>COSE<br>IN GM. | F. A.<br>G | VOL. OF<br>URINE PER<br>24 HR. IN<br>C.C. | SPECIFIC<br>GRAVITY | URINARY GLU-<br>COSE IN GM.<br>BY FOLIN-<br>BERG LUND | URINARY GLU-<br>COSE IN GM.<br>BY<br>POLARISCOPY | FAST-<br>ING BLOOD<br>SUGAR CONCEN-<br>TRATION<br>IN PER CENT | URINARY<br>CREATININE<br>IN GM. | URINARY<br>NITROGEN<br>IN GM. | REMARKS                             |
|--------|------------------|------------------------------------|------------------------------------------|------------|-------------------------------------------|---------------------|-------------------------------------------------------|--------------------------------------------------|---------------------------------------------------------------|---------------------------------|-------------------------------|-------------------------------------|
| June 2 | 69.6             | C-80<br>P-48<br>F-192<br>Cal.-2240 | 127                                      | 1.5        | 1650                                      | 1.009               | 11.93                                                 | 13.8                                             | 0.140                                                         | 1.100                           | 6.4                           |                                     |
| 3      | 69.5             | "                                  | "                                        | "          | 1680                                      | 1.010               | 10.95                                                 | 11.3                                             | 0.148                                                         | 0.965                           | 5.8                           |                                     |
| 4      | 69.3             | "                                  | "                                        | "          | 1580                                      | 1.012               | 16.86                                                 | 17.4                                             | 0.167                                                         | 1.053                           | 6.8                           |                                     |
| 5      | 69.3             | "                                  | "                                        | "          | 1540                                      | 1.012               | 17.19                                                 | 16.6                                             | 0.172                                                         | 0.963                           | 6.4                           |                                     |
| 6      | 69.3             | "                                  | "                                        | "          | 1310                                      | 1.011               | 12.09                                                 | 12.9                                             | 0.148                                                         | 1.007                           | 6.4                           |                                     |
| 7      | 69.3             | "                                  | "                                        | "          | 1860                                      | 1.010               | 11.26                                                 | 11.8                                             | 0.117                                                         | 0.954                           | 7.2                           |                                     |
| 8      | 69.3             | "                                  | "                                        | "          | 1070                                      | 1.010               | 13.72                                                 | 13.7                                             | 0.147                                                         | 0.892                           | 6.4                           |                                     |
| 9      | 69.3             | "                                  | "                                        | "          | 1400                                      | 1.011               | 12.75                                                 | 12.1                                             | 0.138                                                         | 0.900                           | 7.1                           |                                     |
| 10     | 69.3             | "                                  | "                                        | "          | 800                                       | 1.016               | 11.47                                                 | 10.8                                             | 0.142                                                         | 0.748                           | 5.9                           |                                     |
| 11     | 69.3             | "                                  | "                                        | "          | 1860                                      | 1.007               | 2.65                                                  | 2.6                                              | 0.124                                                         | 0.885                           | 6.7                           | Synthalin 0.025 gm.<br>at 8:00 A.M. |
| 12     | 69.3             | "                                  | "                                        | "          | 1790                                      | 1.005               | 2.77                                                  | 2.7                                              | 0.133                                                         | 0.716                           | 6.1                           | Synthalin 0.025 gm.<br>at 8:00 A.M. |
| 13     | 69.3             | "                                  | "                                        | "          | 1540                                      | 1.010               | 1.49                                                  | 1.2                                              | 0.124                                                         | 0.896                           | 5.8                           | Synthalin 0.025 gm.<br>at 8:00 A.M. |

TABLE II—Cont'd

| DATE    | WEIGHT<br>IN KG. | DIET | "Q"<br>OR AVAIL-<br>ABLE GLU-<br>COSE<br>IN GM. | F. A.<br>U | VOL. OF<br>URINE PER<br>24 HR. IN<br>C.C. | SPECIFIC<br>GRAVITY | URINARY GLU-<br>COSE IN GM.<br>BY POLIN-<br>BERGAND | URINARY GLU-<br>COSE IN GM.<br>BY<br>POLARISCOPY | FAST-<br>ING BLOOD<br>SUGAR CONCENTR-<br>ATION<br>IN PER CENT | URINARY<br>CREATININE<br>IN GM. | URINARY<br>NITROGEN<br>IN GM. | REMARKS                             |
|---------|------------------|------|-------------------------------------------------|------------|-------------------------------------------|---------------------|-----------------------------------------------------|--------------------------------------------------|---------------------------------------------------------------|---------------------------------|-------------------------------|-------------------------------------|
| June 13 | 69.3             | "    | "                                               | "          | 1440                                      | 1.006               | 1.16                                                | 1.2                                              | 0.125                                                         | 0.800                           | 5.8                           | Synthalin 0.025 gm.<br>at 8:00 A.M. |
| 15      | 69.3             | "    | "                                               | "          | 1640                                      | 1.006               | 1.29                                                | 1.3                                              | 0.146                                                         | 0.841                           | 7.0                           | Synthalin 0.025 gm.<br>at 8:00 A.M. |
| 16      | 69.3             | "    | "                                               | "          | 1740                                      | 1.006               | 0.56                                                | ---                                              | 0.098                                                         | 0.911                           | 6.4                           | Synthalin 0.025 gm.<br>at 8:00 A.M. |
| 17      | 69.3             | "    | "                                               | "          | 1500                                      | 1.006               | 0.89                                                | ---                                              | 0.121                                                         | 0.858                           | 5.9                           |                                     |
| 18      | 69.0             | "    | "                                               | "          | 1620                                      | 1.011               | 3.66                                                | 3.7                                              | 0.148                                                         | 0.853                           | 6.3                           |                                     |
| 19      | 69.0             | "    | "                                               | "          | 1000                                      | 1.014               | 4.62                                                | 4.5                                              | 0.104                                                         | 0.909                           | 6.1                           |                                     |
| 20      | 69.0             | "    | "                                               | "          | 1120                                      | 1.011               | 4.00                                                | 4.1                                              | 0.102                                                         | 0.896                           | 6.0                           |                                     |
| 21      | 69.0             | "    | "                                               | "          | 1800                                      | 1.004               | 5.19                                                | 5.4                                              | 0.112                                                         | 0.720                           | 6.8                           |                                     |
| 22      | 69.0             | "    | "                                               | "          | 2000                                      | 1.007               | 9.59                                                | 9.3                                              | 0.127                                                         | 0.876                           | 6.3                           |                                     |
| 23      | 68.8             | "    | "                                               | "          | 1500                                      | 1.008               | 8.65                                                | 8.8                                              | 0.128                                                         | 1.020                           | 7.0                           |                                     |
| 24      | 68.4             | "    | "                                               | "          | 1840                                      | 1.009               | 13.19                                               | 13.2                                             | 0.123                                                         | 1.011                           | 5.4                           |                                     |
| 25      | 68.0             | "    | "                                               | "          | 1660                                      | 1.009               | 11.02                                               | 10.0                                             | 0.149                                                         | 1.137                           | 6.0                           |                                     |
| 26      | 68.0             | "    | "                                               | "          | 1200                                      | 1.009               | 7.25                                                | 6.9                                              | 0.121                                                         | 0.987                           | 6.5                           |                                     |
| 27      | 68.0             | "    | "                                               | "          | 2200                                      | 1.005               | 11.75                                               | 12.5                                             | 0.120                                                         | 0.965                           | 6.7                           |                                     |
| 28      | 68.0             | "    | "                                               | "          | 1380                                      | 1.013               | 12.39                                               | 11.6                                             | 0.148                                                         | 0.953                           | 5.6                           |                                     |
| 29      | 67.2             | "    | "                                               | "          | 1100                                      | 1.017               | 15.70                                               | 14.4                                             | 0.143                                                         | 0.974                           | 6.1                           |                                     |

8 and 10 A.M., one third between 12 noon and 2:00 P.M., and one third between 6 and 8 P.M. This diet permits of carbohydrate, 80 gm.; protein, 48 gm.; fat, 192 gm.; glucose, 127 gm.; and cal., 2,240. Four analyses during the experiment indicated that the content of C, P and F of the mixture was constant.

The complete 24-hour specimen of urine was analyzed daily for glucose by the Folin-Berglund method and polariscopy, for nitrogen by the Kjeldahl process and for creatinine by the picramic acid method. The latter was used for a check on the urinary volume. Daily fasting blood sugar determinations were made by the Folin-Wu method.

### RESULTS

When the patient was on the experimental diet, 20 units of insulin before the breakfast and 10 units before the evening meal were necessary to desugarize the urine. When 8 units were given before breakfast and none in the evening, the urinary glucose excretion was at a level that was desirable for study. This dosage was carried throughout the entire experiment. The average daily urinary glucose excretion during the fore-period from April 29 to May 3, inclusive, was 5.76 gm. During the synthalin period from May 4 to 9, inclusive, the average urinary glucose excretion was 1.56 gm. In the after-period, or May 10 to 17, inclusive, the average urinary glucose excretion was 1.90 gm. No toxic effect of the drug was noted.

In this experiment which was carried out the same as Experiment I, the level of glycosuria was within the desirable limits for experimentation without the use of insulin. The average of the daily urinary glucose excretion in the fore-period, June 2 to 10, inclusive, was 13.13 gm. and the nitrogen, 6.5 gm. During the period of the administration of synthalin, June 11 to 16, inclusive, the average daily urinary glucose excretion was 1.65 gm. and the nitrogen, 6.3 gm. In the first after-period, June 17 to 22, inclusive, the average urinary glucose excretion was 4.67 gm. and the nitrogen, 6.2 gm. In the second after-period, June 23 to 29, inclusive, the average daily urinary glucose excretion was 11.42 gm., and the nitrogen was 6.2 gm. No toxic effect of the drug was noted.

### DISCUSSION

The daily administration of 0.025 gm. of synthalin by mouth produced a well-defined reduction in the urinary glucose excretion in this diabetic patient in both experiments. The maximum effect or complete desugarization of the urine was not reached until the fourth day of administration in the first experiment and not until the sixth day in the second experiment. The failure of the level of urinary glucose excretion to return in the after-period to that of the fore-period in the first experiment was probably due to a return of natural tolerance of the patient, brought on by the period of desugarization. This was less evident in the second experiment for the glucose excretion level in the second after-period almost reached that of the fore-period.

Because of the toxic effect on the liver reported by other investigators the use of synthalin was discontinued.

## SUMMARY

The oral administration of synthalin to this diabetic patient completely desugared the urine in four to six days. The effect was sustained over a period of several days.

The author wishes to thank Dr. R. T. Woodyatt for his valuable advice and assistance during these experiments.

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## BASAL METABOLISM OF OLD PEOPLE\*

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## I. INTRODUCTION

IT IS not only for mere scientific interest but is important for practical purposes to determine the normal standard basal metabolism, since it will serve as the basic level when we discuss nutrition problems, such as food requirement, muscular work, and abnormal metabolism. In 1899, Magnus-Levy<sup>1</sup> initiated the work in this field in order to compare the metabolisms of normal and abnormal individuals.

Since then many research workers have published papers on the normal standard metabolism with improved calorimeters and technics. But the difficulty of this research work is that it requires a careful selection of subjects and technics. Especially is it true in determining the normal standard of the old age group.

In their paper Magnus-Levy and Falk presented data which showed the fact that the old age group had a lower metabolic rate than a younger group. Aub and DuBois,<sup>2</sup> Harris Benedict,<sup>3</sup> Boothby and Sandiford,<sup>4</sup> etc., discussed the basal metabolism of the old age group, and they are all in accord that metabolism is lower in older people, as long as they are in a normal condition.

However, it seems to me that their subjects were too few to discuss the fact fully, and in some papers the investigators did not include higher ages.

For the past several years we have carried out a series of experiments to determine reliable data on the normal standard metabolism for the Japanese. This paper is a result of such studies and concerns experimental data collected on the old age group.

## II. EXPERIMENT

*a. Material.*—We have chosen old people of both sexes above the age of fifty years. Due to their higher age, most of them had no special work, but they enjoyed retired life. Persons having definite symptoms of illness at the

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time of study were excluded, though some of them had the so-called senile changes of mild nature occurring in the older people, such as high blood pressure, arteriosclerosis, rheumatism, and chronic nephritis. We regarded these changes to be more or less physiologic accompanying age.

The number which we have examined is 94, consisting of 44 males and 50 females. Their ages were as shown in Table I.

TABLE I

| AGE    | MALE | FEMALE | TOTAL CASES |
|--------|------|--------|-------------|
| 50-59  | 13   | 8      | 21          |
| 60-69  | 15   | 16     | 31          |
| 70-79  | 10   | 18     | 28          |
| 80-89  | 5    | 8      | 13          |
| 90-100 | 1    | --     | 1           |
|        |      |        | 94          |

*b. Method of Study.*—These experiments were carried out during the spring and autumn seasons of 1929 and 1930, in order to have more uniform ranges of dry bulb temperature of 15° C. or 23° C. and dry Kata coefficient of 3.0 or 4.5, both of which were measured near the head of the subjects.

The apparatus used for the experiment was a Benedict's respiration apparatus with cot chamber. Every care was taken and the procedure carried out as the inventors<sup>5</sup> had advised.

### III. RESULT AND DISCUSSION

We have summarized our data in Tables II and III. To determine the surface area, we used Takahira's formula<sup>6</sup> which was derived from actual measurements on Japanese subjects, modifying the Du Bois' height-weight formula,<sup>7</sup> since Japanese have a little different proportion of the body structures than do European races.

$$A = W^{0.427} \times H^{0.718} \times 74.49$$

A-----Surface area in square meters  
 W-----Weight in kilograms  
 74.49-----Constant  
 H-----Height in meters

*a. Comparison of Different Age Groups.*—A comparison of the metabolism in each age group is as follows:

Basal metabolism per square meter per hour (Table IV). The difference between the age group of from fifty to fifty-nine and that of the eighty-year group is 3.99 calories (11 per cent) in man, and 3.65 calories (11 per cent) in woman. The decrease in each ten-year period is from 3 to 5 per cent. The decrease being gradual there is no sudden drop of basal metabolism in any age group.

Heat production per square meter per hour and age have the following correlation coefficient:

|        |                  |
|--------|------------------|
| Male   | -0.5173 ± 0.0745 |
| Female | -0.3804 ± 0.0816 |

Though there is a little different correlation coefficient between male and female, both groups have negative correlation. Calculating with these data we found the prediction equations of heat production based on age to be as follows:

$$\begin{aligned} \text{Male} & \quad H = 42.5042 - 0.1202 A \\ \text{Female} & \quad H = 40.2902 - 0.1086 A \end{aligned}$$

TABLE II

## MALE

| NO.  | AGE  | WEIGHT<br>(KG.) | HEIGHT<br>(CM.) | SURFACE<br>AREA<br>(M) <sup>2</sup> | O <sub>2</sub> CON-<br>SUMED<br>PER HR.<br>(L.) | CO <sub>2</sub> ELIM-<br>INATED<br>PER HR.<br>(L.) | R. Q. | CAL. PER<br>HR. PER<br>SQ. MET. | CAL. PER<br>24 HR. |
|------|------|-----------------|-----------------|-------------------------------------|-------------------------------------------------|----------------------------------------------------|-------|---------------------------------|--------------------|
| 1    | 50   | 49.60           | 154.2           | 1.41                                | 11.24                                           | 8.88                                               | 0.79  | 38.18                           | 1292               |
| 2    | 51   | 50.80           | 157.5           | 1.51                                | 9.95                                            | 9.60                                               | 0.96  | 32.93                           | 1193               |
| 3    | 54   | 47.00           | 146.0           | 1.34                                | 9.71                                            | 8.33                                               | 0.86  | 35.31                           | 1135               |
| 4    | 54   | 39.00           | 142.9           | 1.21                                | 8.31                                            | 6.89                                               | 0.83  | 33.22                           | 965                |
| 5    | 54   | 50.20           | 152.8           | 1.41                                | 10.65                                           | 10.12                                              | 0.96  | 37.74                           | 1277               |
| 6    | 55   | 47.10           | 164.5           | 1.51                                | 10.48                                           | 8.75                                               | 0.83  | 33.59                           | 1217               |
| 7    | 56   | 36.30           | 151.0           | 1.24                                | 9.40                                            | 7.77                                               | 0.81  | 36.48                           | 1086               |
| 8    | 56   | 49.00           | 158.3           | 1.43                                | 10.22                                           | 9.40                                               | 0.92  | 35.39                           | 1215               |
| 9    | 57   | 43.70           | 149.5           | 1.36                                | 10.96                                           | 8.55                                               | 0.78  | 38.49                           | 1256               |
| 10   | 58   | 53.10           | 156.8           | 1.53                                | 11.59                                           | 9.85                                               | 0.85  | 36.84                           | 1253               |
| 11   | 58   | 39.00           | 146.2           | 1.23                                | 9.78                                            | 7.04                                               | 0.72  | 37.36                           | 1103               |
| 12   | 58   | 47.30           | 142.0           | 1.31                                | 9.40                                            | 8.65                                               | 0.92  | 35.50                           | 1116               |
| 13   | 59   | 47.65           | 158.0           | 1.47                                | 11.38                                           | 9.63                                               | 0.85  | 37.63                           | 1217               |
| Ave. | 55.4 | 46.10           | 152.3           |                                     |                                                 |                                                    |       | 36.05                           | 1187               |
| 14   | 60   | 43.00           | 146.5           | 1.33                                | 9.47                                            | 7.39                                               | 0.78  | 33.40                           | 1085               |
| 15   | 60   | 49.50           | 151.5           | 1.49                                | 11.45                                           | 9.55                                               | 0.83  | 37.17                           | 1329               |
| 16   | 60   | 40.00           | 144.0           | 1.28                                | 9.92                                            | 8.96                                               | 0.90  | 38.17                           | 1173               |
| 17   | 63   | 48.70           | 155.0           | 1.46                                | 10.88                                           | 9.46                                               | 0.87  | 36.42                           | 1276               |
| 18   | 63   | 55.20           | 161.0           | 1.57                                | 11.02                                           | 9.16                                               | 0.83  | 33.54                           | 1269               |
| 19   | 64   | 45.90           | 148.0           | 1.33                                | 9.05                                            | 6.60                                               | 0.73  | 32.06                           | 1023               |
| 20   | 64   | 41.60           | 149.2           | 1.28                                | 9.30                                            | 6.60                                               | 0.71  | 34.06                           | 1046               |
| 21   | 65   | 55.50           | 163.0           | 1.61                                | 12.24                                           | 10.33                                              | 0.81  | 36.59                           | 1414               |
| 22   | 65   | 40.25           | 151.5           | 1.33                                | 10.07                                           | 8.37                                               | 0.83  | 36.69                           | 1171               |
| 23   | 66   | 46.00           | 151.8           | 1.35                                | 8.92                                            | 7.22                                               | 0.81  | 31.80                           | 1030               |
| 24   | 66   | 52.85           | 164.5           | 1.62                                | 12.06                                           | 9.90                                               | 0.83  | 36.03                           | 1401               |
| 25   | 66   | 44.22           | 155.0           | 1.40                                | 9.66                                            | 8.66                                               | 0.90  | 33.22                           | 1116               |
| 26   | 66   | 46.90           | 156.0           | 1.46                                | 9.96                                            | 8.07                                               | 0.81  | 32.83                           | 1150               |
| 27   | 66   | 43.20           | 147.5           | 1.31                                | 9.86                                            | 8.31                                               | 0.83  | 35.61                           | 1145               |
| 28   | 68   | 42.55           | 155.0           | 1.38                                | 10.34                                           | 8.38                                               | 0.81  | 36.05                           | 1194               |
| Ave. | 64.1 | 46.40           | 153.5           |                                     |                                                 |                                                    |       | 34.91                           | 1188               |
| 29   | 71   | 52.60           | 161.0           | 1.57                                | 11.31                                           | 9.06                                               | 0.80  | 34.52                           | 1303               |
| 30   | 72   | 33.65           | 156.0           | 1.26                                | 8.11                                            | 6.19                                               | 0.76  | 30.11                           | 901                |
| 31   | 74   | 37.75           | 147.0           | 1.26                                | 9.21                                            | 7.04                                               | 0.76  | 34.73                           | 1050               |
| 32   | 74   | 46.60           | 157.0           | 1.45                                | 10.30                                           | 7.28                                               | 0.77  | 33.84                           | 1178               |
| 33   | 75   | 41.60           | 155.0           | 1.34                                | 9.74                                            | 8.69                                               | 0.91  | 35.88                           | 1154               |
| 34   | 75   | 43.85           | 151.5           | 1.38                                | 9.99                                            | 8.89                                               | 0.89  | 35.56                           | 1178               |
| 35   | 77   | 40.95           | 139.0           | 1.26                                | 8.33                                            | 6.67                                               | 0.80  | 31.78                           | 960                |
| 36   | 78   | 50.00           | 155.5           | 1.48                                | 9.58                                            | 7.74                                               | 0.78  | 30.91                           | 1098               |
| 37   | 79   | 45.69           | 155.5           | 1.43                                | 9.04                                            | 7.59                                               | 0.84  | 30.66                           | 1052               |
| 38   | 79   | 50.70           | 159.5           | 1.52                                | 10.56                                           | 8.47                                               | 0.83  | 33.61                           | 1169               |
| Ave. | 75.4 | 44.30           | 151.0           |                                     |                                                 |                                                    |       | 33.16                           | 1105               |
| 39   | 80   | 35.75           | 151.0           | 1.25                                | 8.38                                            | 5.87                                               | 0.70  | 31.41                           | 942                |
| 40   | 81   | 40.50           | 152.5           | 1.34                                | 8.81                                            | 8.18                                               | 0.84  | 31.89                           | 1134               |
| 41   | 83   | 48.15           | 167.0           | 1.54                                | 9.05                                            | 7.31                                               | 0.81  | 28.28                           | 1046               |
| 42   | 83   | 39.60           | 152.5           | 1.29                                | 9.08                                            | 7.81                                               | 0.86  | 33.53                           | 1062               |
| 43   | 89   | 59.30           | 153.0           | 1.54                                | 10.67                                           | 8.43                                               | 0.79  | 33.18                           | 1226               |
| 44   | 93   | 44.50           | 154.5           | 1.40                                | 9.87                                            | 8.22                                               | 0.83  | 34.10                           | 1146               |
| Ave. | 84.8 | 44.63           | 145.1           |                                     |                                                 |                                                    |       | 22.06                           | 1075               |

This fact indicates that the basal metabolism of the human body decreases inversely to the increase of ages over fifty years old. If we consider the basal metabolism as the index of vitality, we think this decrease is a reasonable phenomenon.

TABLE III

## FEMALE

| NO.  | AGE  | WEIGHT<br>(KG.) | HEIGHT<br>(CM.) | SURFACE<br>AREA<br>(M) <sup>2</sup> | O <sub>2</sub> CON-<br>SUMED<br>PER HR. | CO <sub>2</sub> ELIM-<br>INATED<br>PER HR. | R. Q. | CAL. PER<br>HR. PER<br>SQ. MET. | CAL. PER<br>24 HR. |
|------|------|-----------------|-----------------|-------------------------------------|-----------------------------------------|--------------------------------------------|-------|---------------------------------|--------------------|
| 1    | 54   | 47.60           | 144.1           | 1.32                                | 9.08                                    | 7.99                                       | 0.88  | 33.70                           | 1063               |
| 2    | 54   | 41.20           | 141.8           | 1.23                                | 7.83                                    | 6.81                                       | 0.87  | 31.09                           | 918                |
| 3    | 55   | 50.30           | 139.5           | 1.32                                | 8.41                                    | 7.65                                       | 0.91  | 31.43                           | 996                |
| 4    | 56   | 42.40           | 144.6           | 1.26                                | 8.61                                    | 7.07                                       | 0.82  | 32.98                           | 997                |
| 5    | 57   | 55.00           | 139.6           | 1.37                                | 10.51                                   | 9.46                                       | 0.90  | 37.77                           | 1242               |
| 6    | 58   | 40.80           | 152.1           | 1.34                                | 9.91                                    | 8.92                                       | 0.90  | 36.44                           | 1171               |
| 7    | 58   | 33.80           | 149.5           | 1.21                                | 9.13                                    | 8.01                                       | 0.87  | 36.88                           | 1071               |
| 8    | 59   | 42.00           | 144.5           | 1.29                                | 8.43                                    | 7.88                                       | 0.93  | 32.42                           | 1004               |
| Ave. | 56.4 | 44.14           | 144.5           |                                     |                                         |                                            |       | 34.02                           | 1058               |
| 9    | 60   | 47.30           | 147.5           | 1.39                                | 9.51                                    | 8.15                                       | 0.86  | 33.33                           | 1112               |
| 10   | 62   | 42.30           | 137.5           | 1.26                                | 9.26                                    | 7.75                                       | 0.84  | 35.65                           | 1078               |
| 11   | 62   | 41.30           | 140.5           | 1.26                                | 7.64                                    | 6.71                                       | 0.88  | 29.69                           | 898                |
| 12   | 62   | 40.90           | 142.0           | 1.23                                | 7.98                                    | 7.26                                       | 0.91  | 31.96                           | 945                |
| 13   | 65   | 50.00           | 136.0           | 1.32                                | 9.04                                    | 8.24                                       | 0.91  | 33.76                           | 1071               |
| 14   | 65   | 37.80           | 143.0           | 1.20                                | 8.43                                    | 6.99                                       | 0.83  | 33.97                           | 979                |
| 15   | 66   | 34.30           | 147.0           | 1.17                                | 8.42                                    | 6.65                                       | 0.79  | 34.47                           | 967                |
| 16   | 67   | 43.10           | 137.5           | 1.22                                | 8.34                                    | 7.84                                       | 0.94  | 34.01                           | 996                |
| 17   | 67   | 29.70           | 139.0           | 1.10                                | 7.17                                    | 6.32                                       | 0.88  | 31.95                           | 843                |
| 18   | 67   | 32.30           | 141.0           | 1.33                                | 7.96                                    | 6.71                                       | 0.84  | 29.04                           | 927                |
| 19   | 67   | 43.30           | 140.5           | 1.29                                | 8.40                                    | 7.46                                       | 0.89  | 31.98                           | 990                |
| 20   | 67   | 38.10           | 136.5           | 1.22                                | 8.70                                    | 7.41                                       | 0.85  | 34.73                           | 1017               |
| 21   | 67   | 42.90           | 143.0           | 1.29                                | 8.89                                    | 7.53                                       | 0.85  | 33.51                           | 1038               |
| 22   | 69   | 40.10           | 137.0           | 1.24                                | 9.20                                    | 8.26                                       | 0.90  | 36.53                           | 1087               |
| 23   | 69   | 42.70           | 148.0           | 1.34                                | 8.71                                    | 7.60                                       | 0.87  | 31.76                           | 1022               |
| 24   | 69   | 37.70           | 142.0           | 1.24                                | 8.96                                    | 7.39                                       | 0.83  | 34.15                           | 1016               |
| Ave. | 65.7 | 40.28           | 141.1           |                                     |                                         |                                            |       | 33.15                           | 999                |
| 25   | 72   | 38.00           | 143.5           | 1.25                                | 7.67                                    | 6.64                                       | 0.87  | 29.96                           | 899                |
| 26   | 73   | 38.80           | 137.5           | 1.22                                | 8.93                                    | 7.88                                       | 0.88  | 35.84                           | 1050               |
| 27   | 73   | 33.50           | 144.0           | 1.19                                | 8.07                                    | 6.98                                       | 0.87  | 33.11                           | 946                |
| 28   | 74   | 40.40           | 141.0           | 1.27                                | 9.05                                    | 7.33                                       | 0.81  | 34.27                           | 1045               |
| 29   | 75   | 31.20           | 139.5           | 1.10                                | 6.01                                    | 5.12                                       | 0.85  | 26.54                           | 701                |
| 30   | 76   | 35.50           | 140.0           | 1.19                                | 8.30                                    | 7.18                                       | 0.87  | 34.08                           | 973                |
| 31   | 76   | 40.95           | 145.0           | 1.30                                | 8.15                                    | 7.19                                       | 0.88  | 30.72                           | 958                |
| 32   | 76   | 46.30           | 136.5           | 1.31                                | 9.04                                    | 7.96                                       | 0.88  | 33.82                           | 1063               |
| 33   | 76   | 40.40           | 150.0           | 1.33                                | 9.25                                    | 7.41                                       | 0.79  | 29.98                           | 957                |
| 34   | 76   | 35.15           | 138.5           | 1.15                                | 8.45                                    | 7.35                                       | 0.87  | 35.91                           | 991                |
| 35   | 77   | 46.90           | 146.5           | 1.35                                | 8.14                                    | 6.67                                       | 0.82  | 29.09                           | 942                |
| 36   | 77   | 34.70           | 142.0           | 1.18                                | 8.31                                    | 7.15                                       | 0.86  | 44.33                           | 972                |
| 37   | 77   | 40.55           | 146.0           | 1.30                                | 8.25                                    | 6.85                                       | 0.83  | 30.70                           | 958                |
| 38   | 77   | 33.20           | 136.5           | 1.13                                | 8.73                                    | 6.52                                       | 0.75  | 35.74                           | 969                |
| 39   | 77   | 39.90           | 145.0           | 1.28                                | 9.30                                    | 7.81                                       | 0.84  | 35.23                           | 1082               |
| 40   | 78   | 39.30           | 138.0           | 1.23                                | 7.93                                    | 7.35                                       | 0.92  | 31.89                           | 942                |
| 41   | 78   | 34.70           | 145.5           | 1.23                                | 8.66                                    | 7.39                                       | 0.85  | 34.22                           | 1011               |
| 42   | 78   | 35.00           | 141.0           | 1.18                                | 6.90                                    | 6.27                                       | 0.91  | 28.85                           | 817                |
| Ave. | 75.9 | 38.03           | 142.0           |                                     |                                         |                                            |       | 31.90                           | 960                |
| 43   | 80   | 40.80           | 147.0           | 1.28                                | 7.71                                    | 6.72                                       | 0.87  | 29.65                           | 904                |
| 44   | 82   | 31.90           | 138.0           | 1.13                                | 8.00                                    | 5.85                                       | 0.73  | 33.37                           | 905                |
| 45   | 82   | 47.00           | 140.0           | 1.34                                | 8.78                                    | 7.14                                       | 0.81  | 31.54                           | 1014               |
| 46   | 83   | 37.30           | 148.0           | 1.27                                | 7.67                                    | 6.76                                       | 0.88  | 29.61                           | 902                |
| 47   | 83   | 31.70           | 131.0           | 1.08                                | 6.95                                    | 5.04                                       | 0.73  | 29.61                           | 687                |
| 48   | 83   | 32.80           | 127.0           | 1.07                                | 6.89                                    | 6.09                                       | 0.88  | 31.55                           | 810                |
| 49   | 84   | 28.95           | 130.5           | 1.03                                | 6.08                                    | 5.08                                       | 0.83  | 28.55                           | 706                |
| 50   | 86   | 30.00           | 138.5           | 1.10                                | 6.57                                    | 5.91                                       | 0.90  | 29.44                           | 777                |
| Ave. | 83.0 | 35.06           | 137.5           |                                     |                                         |                                            |       | 30.42                           | 851                |



TABLE IV

| AGE   | MAN        | WOMAN      |
|-------|------------|------------|
| 50-59 | 36.05 cal. | 34.02 cal. |
| 60-69 | 34.91 cal. | 33.15 cal. |
| 70-79 | 33.16 cal. | 31.90 cal. |
| 80-   | 32.06 cal. | 30.42 cal. |

*b. Sex Difference.*—As Table IV shows, we noticed some difference between the two sexes. It is natural that a female requires less calories per day than a male, as the former usually is smaller in size. However, even when we compare the basal metabolism with body surface area, we notice the same differ-

Calories per Hour per Square Meter

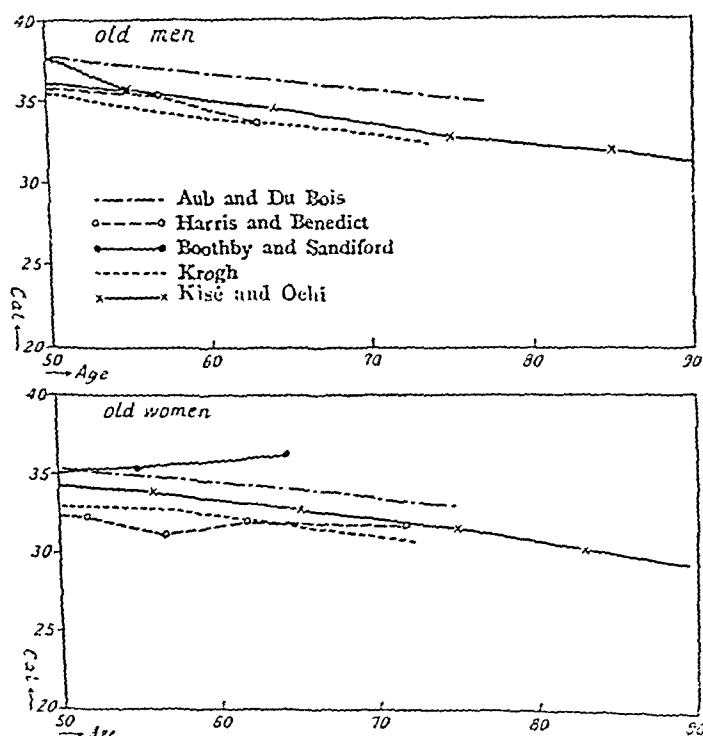


Fig. 1.

ence which is effected, since the constitution and activities of the bodies of male and female are different. In the old age group we found the average value of males to be 34.46 cal. per unit, that of females to be 32.62 cal. This difference can be seen in every age group with an average difference of 5 per cent. This value is coincident with the data reported by Magnus-Levy<sup>1</sup> and Benedict-Emmes.<sup>5</sup>

*c. Comparison Between Young and Old Age Groups.*—Takahira<sup>6</sup> first reported reliable data on the normal standard of basal metabolism of the Japanese. He dealt with the age group between twenty and fifty, and showed the averages to be as follows:

|        |                   |
|--------|-------------------|
| Male   | $37.33 \pm 0.182$ |
| Female | $33.84 \pm 0.301$ |

Our results in the old age groups are:

|        |                   |
|--------|-------------------|
| Male   | 34.46 $\pm$ 0.247 |
| Female | 32.62 $\pm$ 0.242 |

By a comparison of these two averages we find that the old age group has a lower basal metabolism by 6 per cent. The difference between the two sexes is 9 per cent among the young group, while that of the older group is 5 per cent. Here we notice that the difference of metabolism between the sexes becomes smaller with the increase of ages.

*d. Comparison With Other Standards.*—Aub and Du Bois<sup>2</sup> reported that people of from seventy-seven to eighty-three years had a basal metabolism of 35.1 cal., i.e., 10 per cent lower than that of adults. Harris and Benedict<sup>3</sup> reported the basal metabolism of the sixty to sixty-four group as 34.8 cal., i.e., 9 per cent lower than the young group. According to Bailey's data<sup>10</sup> the basal metabolism in the sixty to sixty-five year group is 5 per cent lower than that of the younger group. In the data reported by Boothby and Sandiford<sup>11</sup> the metabolism of a healthy man, aged sixty years, was 37.4 cal., and calculation showed that it was 10 per cent lower than that of their healthy younger group. Krogh<sup>12</sup> modified the Aub-Du Bois standard for ages fifteen to seventy-five and recommended a uniform reduction of 6 per cent from the standard.

We cannot compare these data with each other directly, since the groupings of the ages are different. Curves are drawn for graphic comparison. (Fig. 1.)

Our curve on male groups almost agrees with that of Harris and Benedict in the period of from fifty to fifty-nine, but in female groups it differs a little, and it lies rather near to the Aub and Du Bois' data. In general, our curve drawn with the data of the Japanese resembles that of Krogh, though our data showed less difference between the sexes.

#### SUMMARY

Basal metabolism was measured upon 94 normal Japanese subjects, whose ages ranged from fifty to ninety-three years. It was found that the metabolism of the aged is lower than that of younger people, and it decreases gradually without a sudden drop in any age group. The rate of decrease in each ten-year period is about 3 to 5 per cent. We found that the difference in rates between the sexes in the old age groups is smaller than in the younger groups. Comparing with the other standards, the curve drawn on our data agrees very closely with that of Krogh's modification.

The authors desire to express their appreciation to Professor Yoshio Kusama for much helpful advice and criticism throughout this work.

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## THE BLOOD PICTURE IN ORAL INFECTION\*

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THE data which form the basis of this report are the determination of hemoglobin percentage and the blood counts in the same cases of oral infection before and after treatment. These data were abstracted from the papers by Adams, Bryan et al., Bryant and Plevitzky, Burns, Harvey et al., Ivy, Logan, and Toren.

In the original papers only the percentages of the several kinds of leucocytes were given. From these percentages and the white blood cell count, we have calculated the absolute numbers of the several leucocyte types in the cubic millimeter.

Certain shortcomings must be pointed out in order to emphasize the tentativeness of any conclusions reached in this report and in order to point out certain precautions which should attend the collection of fresh data. The clinical condition of the patient, both general and local, should be clearly stated. The indefiniteness of our title is necessitated by the indefiniteness of several of the authors whose papers furnished the data. In the selection of cases, serious and competent effort should be made to limit the observations to patients who are suffering only from one or the other of the types of oral infection chosen for study. Usually no mention was made of the technic used to determine the hemoglobin percentage. This is necessary to reduce observations to a common term before valid comparisons can be made. We have *presumed* that the same technic was used after as before treatment. If this was done, then a comparison is legitimate, although we can form no idea of the absolute amount of hemoglobin present. The time of day at which the white blood cell count was made was generally not mentioned. The possible effects of meals or of exercise apparently were disregarded. A standard procedure is desirable, e.g., the count should be made routinely in the morning before arising and on a fasting stomach. The erythrocyte, leucocyte, and differential counts made before and after treatment in any given case should be made by the same individual, to minimize the "per-

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sonal equation." The differential count should be expressed not only in percentages but also in absolute numbers.

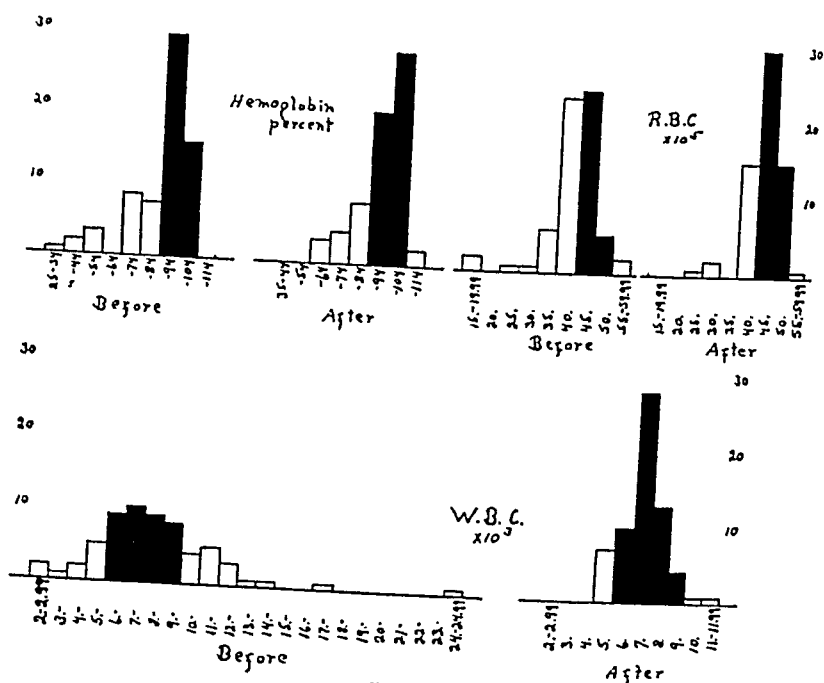


Chart 1.

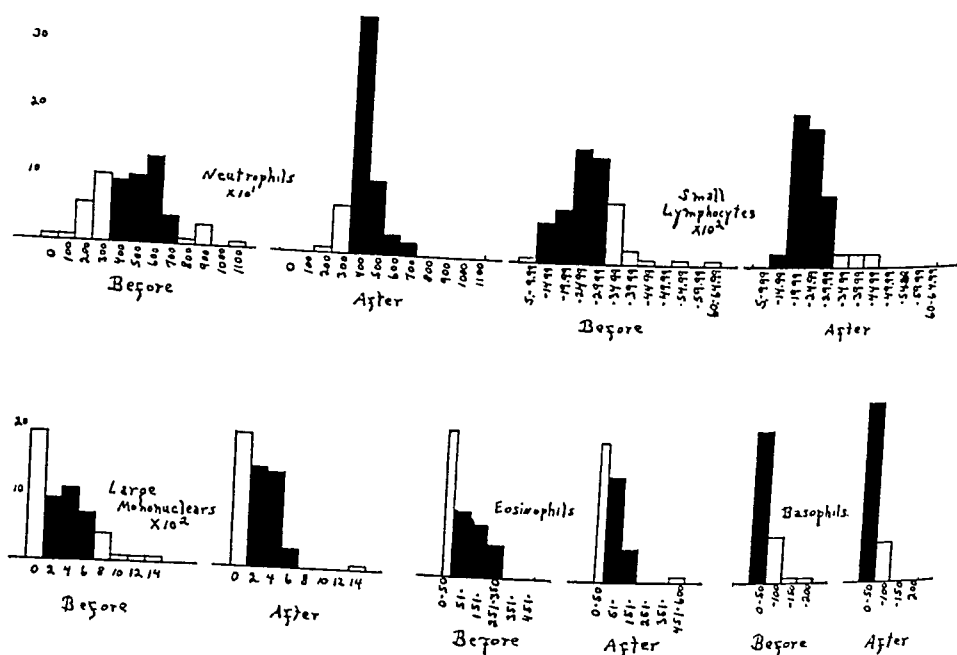


Chart 2.

The data collected are presented in Table I and Figs. 1 to 3. The cases falling within the normal ranges are represented by the blocked-in columns:

those falling outside the normal ranges, by the outlined columns. The number of cases represented by each column is indicated by the numbers at the left. Inasmuch as the number of cases used in constructing the *before treatment* graphs is the same as the number used in constructing the *after treatment* graphs, it was unnecessary to translate these numbers into percentages to permit comparisons.

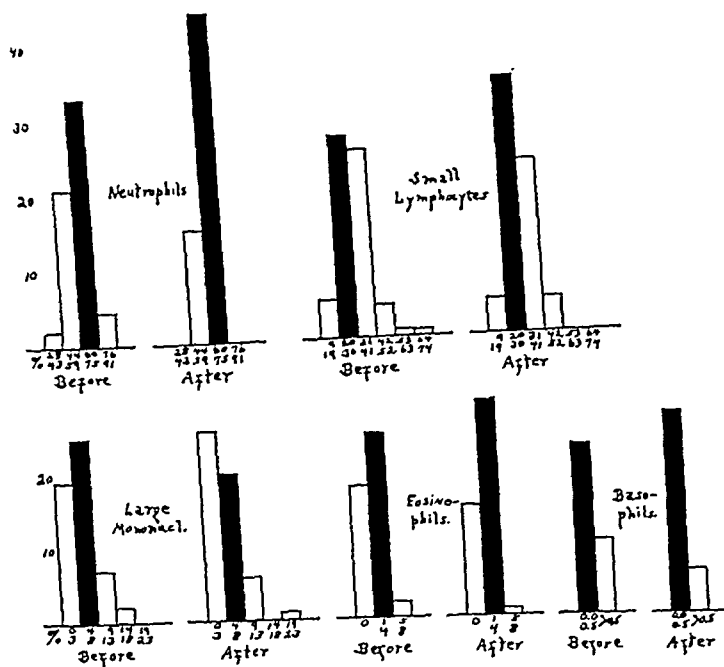


Chart 3.

### CONCLUSIONS

These are subject to the limitations and criticisms mentioned at the beginning of this paper.

1. Most cases of oral infection do not induce material changes in the blood picture.
2. In some cases, however, departures from normal are seen. Some of these departures are marked. The outstanding changes, when they occur, are:
  - a. An anemia manifested by a lowering of both the percentage of hemoglobin and of the number of erythrocytes.
  - b. Either a leucopenia or a hyperleucocytosis. In the present series, the tendency toward a hyperleucocytosis is slightly the stronger.
  - c. The number and percentage of neutrophils may be above or below normal. In this series, subnormal values appear the more frequently.
  - d. When the small lymphocytes show any change, it is usually in the direction of an increase in their number and percentage. Thus there may be both an absolute and a relative lymphocytosis.
  - e. The number and percentage of large mononuclears, and eosinophiles often run low.

TABLE I

TABLE I

|                    | RANGE OF READINGS |         |         |         | NUMBER READINGS RECORDED |    | NUMBER READINGS IN NORMAL RANGE |    | % TOTAL READINGS IN NORMAL RANGE |    |
|--------------------|-------------------|---------|---------|---------|--------------------------|----|---------------------------------|----|----------------------------------|----|
|                    | BEFORE            |         | AFTER   |         |                          |    |                                 |    |                                  |    |
|                    |                   | DIFF.   |         | DIFF.   |                          |    |                                 |    |                                  |    |
|                    |                   |         |         |         | B.                       | A. | B.                              | A. | B.                               | A. |
| Hb. per cent       | 30                | 70%     | 58      | 52%     | 65                       | 65 | 44                              | 48 | 68                               | 74 |
| Total R.B.C.       | 100               |         | 110     |         |                          |    |                                 |    |                                  |    |
|                    | 1600000           |         | 2700000 |         |                          |    |                                 |    |                                  |    |
| Total W.B.C.       | 5840000           | 4240000 | 5600000 | 2900000 | 64                       | 64 | 29                              | 45 | 45                               | 70 |
|                    | 2250              |         | 5200    |         |                          |    |                                 |    |                                  |    |
|                    | 24400             | 22150   | 11800   | 6600    | 63                       | 64 | 36                              | 55 | 57                               | 86 |
| Neutrophiles       | 560               |         | 2940    |         |                          |    |                                 |    |                                  |    |
|                    | 11220             | 10660   | 7550    | 4610    | 59                       | 59 | 36                              | 51 | 61                               | 86 |
| Small lymphocytes  | 950               |         | 1152    |         |                          |    |                                 |    |                                  |    |
|                    | 6460              | 5510    | 4032    | 2880    | 62                       | 63 | 47                              | 57 | 76                               | 90 |
| Large mononuclears | 0                 |         | 0       |         |                          |    |                                 |    |                                  |    |
|                    | 1592              | 1592    | 1554    | 1554    | 53                       | 53 | 27                              | 32 | 51                               | 60 |
| Eosinophiles       | 0                 |         | 0       |         |                          |    |                                 |    |                                  |    |
|                    | 348               | 348     | 539     | 539     | 45                       | 43 | 23                              | 21 | 51                               | 49 |
| Basophiles         | 0                 |         | 0       |         |                          |    |                                 |    |                                  |    |
|                    | 174               | 174     | 98      | 98      | 33                       | 33 | 23                              | 27 | 70                               | 82 |

f. The number and percentage of basophiles may run slightly above the normal range.

The numbers of large mononuclears, eosinophiles and basophiles actually noted in doing a "blood count" are so small that it would not be unlikely if later and more extensive studies reversed the statements listed above as e. and f.

3. Following treatment the observed values usually tend to fall within the normal range.

4. This fact supports the views: (a) that the dental infection contributed to the abnormal blood picture, and (b) that the dental treatment applied was more or less successful in combating the infection.

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# THE EFFECT OF VARIOUS COLLOIDAL AND CRYSTALLOIDAL METALLIC COMPOUNDS IN NUTRITIONAL ANEMIA OF THE RAT\*

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KEIL and Nelson<sup>1</sup> have recently made an extensive study of the rôle of copper and certain other elements and amino acids in the regeneration of hemoglobin. This work constituted an elaboration and verification of earlier data by Hart, Steenbock, and coworkers.<sup>2</sup> Goerner<sup>3</sup> states that salts of manganese, as well as of copper, are capable of increasing the hemoglobin of anemic animals when these salts are added to salts of iron. However, he observed that colloidal solutions of manganese and copper in the presence of colloidal solutions of iron have no hematopoietic effect. Myers and Beard<sup>4</sup> found that higher doses of zinc and magnesium retarded blood regeneration.

The experiments recorded in this paper were instituted in order to answer certain questions: First, does manganese act like copper in hematopoiesis? Second, are colloidal Fe and Cu utilized in hemoglobin building? Third, will Fe salts injected intraperitoneally cause regeneration in anemic animals? Fourth, what effect do Zn and Mg salts have on the development of anemia? Fifth, what are the minimum amounts of Fe and Cu required for regeneration of hemoglobin? Sixth, to what extent can different compounds of Cu be utilized in hematopoiesis?

## EXPERIMENTAL

All of the experiments were performed on rats. The milk used for the production of anemia was collected directly into glass containers from pure bred Holstein cows, in order to avoid contamination with copper. The salts were examined spectrographically by a Hilger quartz prism spectrograph and

TABLE I  
DIET: WHOLE MILK COLLECTED IN GLASS

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 14.6                              | 100.0                       | 16                   |
| 2                | 10.6                              | 72.6                        | 16                   |
| 4                | 6.2                               | 42.4                        | 16                   |
| 6                | 5.0                               | 34.2                        | 15                   |
| 8                | 3.8                               | 26.0                        | 13                   |

shown to be pure. Carbon electrodes were used in the spectrographic examination. Hemoglobin content was determined by the Newcomer method. The animals were bled by the tail.

It is evident from the data in Table I that the consumption of the whole milk used in these experiments produces a very marked anemia. Data have

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also been obtained which show that anemia results even though iron salts are added to this milk. On the other hand, animals receiving ordinary market milk plus iron salts develop anemia much more slowly. This is due to the fact that ordinary market milk contains more copper than milk collected directly into glass. The amount of copper in market milk is variable.

TABLE II  
EFFECT OF IRON AND COPPER SALTS ON ANEMIA

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 3.7                               | 100.0                       | 12                   |
| 2                | 9.6                               | 259.3                       | 12                   |
| 4                | 13.0                              | 351.0                       | 12                   |
| 6                | 15.2                              | 410.0                       | 12                   |
| 8                | 14.9                              | 402.5                       | 12                   |

Table II shows that copper is very potent in hematopoiesis. The animals were made anemic on whole milk, and when the hemoglobin had fallen to 3.7 gm. per 100 c.c., 0.50 mg. of Fe as  $\text{FeCl}_3$  and 0.05 mg. Cu as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  were added to the basal diet daily. When the rats were eight weeks of age the average hemoglobin was 402.5 per cent of that at the anemic level.

TABLE III  
EFFECT OF IRON AND MANGANESE ON ANEMIA

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 6.8                               | 100.0                       | 10                   |
| 2                | 6.6                               | 97.0                        | 10                   |
| 4                | 5.2                               | 76.5                        | 8                    |
| 6                | 4.8                               | 70.6                        | 6                    |

Table III shows that the addition of 0.50 mg. of Fe as  $\text{FeCl}_3$  and 0.10 mg. Mn as  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  daily to the milk failed to stimulate regeneration of hemoglobin. Manganese therefore cannot replace copper.

TABLE IV  
EFFECT OF COLLOIDAL IRON AND COPPER SULPHATE ON ANEMIA

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 4.5                               | 100.0                       | 10                   |
| 2                | 10.6                              | 235.5                       | 10                   |
| 4                | 11.0                              | 244.1                       | 10                   |
| 6                | 14.7                              | 326.2                       | 10                   |
| 8                | 15.0                              | 333.0                       | 10                   |

Table IV demonstrates that colloidal iron can be utilized in the building of hemoglobin. The colloidal iron was prepared from copper-free  $\text{FeCl}_3$  solution. The latter solution was added to boiling Cu-free water, then dialyzed until free from dialyzable iron. After the animals had developed anemia, 0.50 mg. Fe as colloidal iron and 0.05 mg. of copper as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  were added to the milk diet. The same results were obtained by the injection of the colloidal iron intraperitoneally.



TABLE V  
EFFECT OF FERRIC CHLORIDE AND COLLOIDAL COPPER ON ANEMIA

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 4.6                               | 100.0                       | 10                   |
| 2                | 10.0                              | 217.3                       | 10                   |
| 4                | 13.5                              | 293.5                       | 10                   |
| 6                | 15.2                              | 330.4                       | 10                   |

The data in Table V show that colloidal copper is also utilized in hemoglobin formation. The copper was nondialyzable. The animals received milk until they were anemic, and then 0.50 mg. of Fe as  $\text{FeCl}_3$  and 0.05 mg. Cu as colloidal copper were added.

TABLE VI  
EFFECT OF COLLOIDAL IRON AND COLLOIDAL COPPER ON ANEMIA

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 4.0                               | 100.0                       | 12                   |
| 2                | 7.8                               | 195.0                       | 12                   |
| 4                | 10.6                              | 265.0                       | 12                   |
| 6                | 12.0                              | 300.0                       | 12                   |
| 8                | 12.9                              | 322.5                       | 12                   |
| 10               | 13.8                              | 345.0                       | 12                   |
| 12               | 14.6                              | 365.0                       | 12                   |
| 14               | 15.1                              | 377.5                       | 12                   |

Table VI shows that colloidal Fe and Cu are utilized for the construction of hemoglobin. The animals developed anemia, and then 0.50 mg. of Fe as colloidal Fe and 0.05 mg. of Cu as colloidal Cu were fed daily. The same results were obtained by injection of the iron and copper intraperitoneally.

TABLE VII  
EFFECT OF INTRAPERITONEAL INJECTION OF IRON SALTS ON ANEMIA

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| Ferric Citrate   |                                   |                             |                      |
| 0                | 6.2                               | 100.0                       | 10                   |
| 2                | 10.0                              | 161.4                       | 10                   |
| 4                | 11.4                              | 183.9                       | 10                   |
| 6                | 11.6                              | 187.1                       | 10                   |
| 8                | 11.0                              | 177.5                       | 10                   |
| 10               | 9.5                               | 153.2                       | 10                   |
| Ferric Chloride  |                                   |                             |                      |
| 0                | 6.8                               | 100.0                       | 18                   |
| 2                | 9.6                               | 141.2                       | 18                   |
| 4                | 10.2                              | 150.0                       | 18                   |
| 6                | 11.0                              | 161.7                       | 17                   |
| 8                | 11.2                              | 164.6                       | 17                   |
| 10               | 10.7                              | 157.2                       | 16                   |
| 12               | 9.2                               | 135.3                       | 16                   |

Table VII shows that Fe as citrate when injected intraperitoneally results in a temporary stimulation of hemoglobin formation. This experiment was performed in order to ascertain if copper was concerned in the absorption of Fe. Copper may play some part in the absorption of iron, but the data show

clearly that the rôle of Cu in hemoglobin regeneration cannot be explained on this basis alone.  $\text{FeCl}_3$  acts like iron citrate although it causes necrosis.  $\text{FeCl}_3$  in glycerol, however, does not have this deleterious effect when injected. The Fe as citrate and chloride was administered at a level of 0.50 mg. of Fe daily.

TABLE VIII  
EFFECT OF ZINC AND MAGNESIUM SALTS ON ANEMIA

| TIME<br>IN WEEKS                    | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|-------------------------------------|-----------------------------------|-----------------------------|----------------------|
| $\text{ZnCl}_2$ and $\text{FeCl}_3$ |                                   |                             |                      |
| 0                                   | 12.5                              | 100.0                       | 6                    |
| 2                                   | 11.0                              | 87.9                        | 6                    |
| 4                                   | 9.4                               | 75.2                        | 6                    |
| 5                                   | 8.7                               | 69.6                        | 6                    |
| $\text{MgCl}_2$ and $\text{FeCl}_3$ |                                   |                             |                      |
| 0                                   | 12.3                              | 100.0                       | 6                    |
| 2                                   | 10.8                              | 87.8                        | 6                    |
| 4                                   | 8.7                               | 70.8                        | 6                    |
| 5                                   | 7.5                               | 61.0                        | 6                    |
| $\text{FeCl}_3$                     |                                   |                             |                      |
| 0                                   | 13.1                              | 100.0                       | 6                    |
| 2                                   | 11.4                              | 87.0                        | 6                    |
| 4                                   | 9.7                               | 74.1                        | 6                    |
| 5                                   | 8.8                               | 67.2                        | 6                    |

The data in Table VIII show no effect of Zn and Mg on the development of anemia. The rate of fall of hemoglobin was the same with or without these elements. Two-tenths milligram of Zn as  $\text{ZnCl}_2$  and 0.2 mg. of Mg as  $\text{MgCl}_2$  were fed daily, together with 0.50 mg. Fe as  $\text{FeCl}_3$ . Myers and Beard<sup>4</sup> have

TABLE IX  
EFFECT OF INTRAPERITONEAL INJECTION OF 0.002 MG. CU AS  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  DAILY

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 7.4                               | 100.0                       | 12                   |
| 2                | 11.8                              | 159.5                       | 12                   |
| 4                | 14.0                              | 189.2                       | 12                   |
| 6                | 14.8                              | 200.0                       | 12                   |
| 8                | 15.0                              | 202.7                       | 12                   |

TABLE X  
EFFECT OF INTRAPERITONEAL INJECTION OF 0.10 MG. FE AS  $\text{FeCl}_3$ , DAILY

| TIME<br>IN WEEKS | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|------------------|-----------------------------------|-----------------------------|----------------------|
| 0                | 6.0                               | 100.0                       | 12                   |
| 2                | 8.9                               | 148.3                       | 12                   |
| 4                | 10.4                              | 173.3                       | 12                   |
| 6                | 11.8                              | 196.6                       | 12                   |
| 8                | 13.2                              | 220.0                       | 12                   |
| 10               | 14.3                              | 238.3                       | 12                   |
| 12               | 15.1                              | 251.6                       | 12                   |

stated that larger doses of Zn and Mg retard blood regeneration. We therefore believed anemia might develop more rapidly on milk plus  $\text{FeCl}_3$  if these elements were included. The data show this is not the case.

Table IX shows that normal regeneration of hemoglobin is obtained by 0.002 mg. Cu as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  injected intraperitoneally daily. The animals received milk *ad lib.* and orally 0.50 mg. of Fe as  $\text{FeCl}_3$  daily. This is the smallest amount of Cu that would cause regeneration of hemoglobin to the normal level.

Table X illustrates that regeneration of hemoglobin to the normal level is obtained by injection intraperitoneally of 0.10 mg. Fe as colloidal ferric hydroxide together with 0.002 mg. Cu as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ . Smaller amounts of Fe failed to cause stimulation of hemoglobin formation to the normal level.

TABLE XI  
EFFECT OF INSOLUBLE COPPER COMPOUNDS ON ANEMIA

| TIME<br>IN WEEKS              | AVERAGE HB.<br>(GM. PER 100 C.C.) | PER CENT OF<br>ORIGINAL HB. | NUMBER OF<br>ANIMALS |
|-------------------------------|-----------------------------------|-----------------------------|----------------------|
| Copper Sulphide 0.02 Mg. Cu   |                                   |                             |                      |
| 0                             | 6.0                               | 100.0                       | 6                    |
| 2                             | 11.0                              | 183.3                       | 6                    |
| 4                             | 12.3                              | 205.0                       | 6                    |
| 6                             | 11.5                              | 191.6                       | 6                    |
| 8                             | 11.7                              | 195.0                       | 6                    |
| 10                            | 12.0                              | 200.0                       | 6                    |
| 12                            | 13.4                              | 223.3                       | 6                    |
| 14                            | 14.8                              | 246.6                       | 6                    |
| Copper Hydroxide 0.005 Mg. Cu |                                   |                             |                      |
| 0                             | 6.1                               | 100.0                       | 6                    |
| 2                             | 11.2                              | 183.5                       | 6                    |
| 4                             | 12.0                              | 196.7                       | 6                    |
| 6                             | 12.8                              | 210.0                       | 6                    |
| 8                             | 14.1                              | 231.2                       | 6                    |
| 10                            | 13.8                              | 226.3                       | 6                    |
| 12                            | 14.2                              | 232.8                       | 6                    |
| 14                            | 15.0                              | 246.0                       | 6                    |
| Cuprous Oxide 0.01 Mg. Cu     |                                   |                             |                      |
| 0                             | 4.2                               | 100.0                       | 6                    |
| 2                             | 8.8                               | 209.5                       | 6                    |
| 4                             | 11.9                              | 283.5                       | 6                    |
| 6                             | 13.6                              | 323.8                       | 6                    |
| 8                             | 14.6                              | 347.0                       | 6                    |
| 9                             | 14.8                              | 352.0                       | 6                    |
| Cuprous Iodide 0.01 Mg. Cu    |                                   |                             |                      |
| 0                             | 5.0                               | 100.0                       | 6                    |
| 2                             | 9.4                               | 188.0                       | 6                    |
| 4                             | 11.3                              | 226.0                       | 6                    |
| 6                             | 13.5                              | 270.0                       | 6                    |
| 8                             | 14.5                              | 290.0                       | 6                    |
| 9                             | 15.1                              | 302.0                       | 6                    |

The data in Table XI show that the animal body can utilize various copper compounds in the production of hemoglobin, even though these salts be very insoluble. The character of the anion apparently has no effect on the utilization of the copper.

#### SUMMARY

1. Manganese cannot replace Cu in the synthesis of hemoglobin.
2. Colloidal Fe and Cu are utilized in hematopoiesis.
3. Although the intraperitoneal injection of Fe salts into anemic animals

causes a temporary rise in hemoglobin, it is evident that the main function of Cu is not in the absorption of iron.

4. Zinc and Mg have no effect in the development of anemia.

5. Two-thousandths (0.002) mg. Cu as  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 0.10 mg. Fe as colloidal  $\text{Fe}(\text{OH})_3$  are the minimum amounts of these elements that will cause a regeneration of the hemoglobin to a normal level in the anemic rat.

6.  $\text{Cu}_2\text{O}$ , CuS,  $\text{Cu}(\text{OH})_2$ , and CuI are readily utilized by the anemic rat in hemoglobin building. The sulphide is less efficient than the other salts employed.

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### EXPERIMENTS IN THE USE OF A MINERAL WATER IN THE MANAGEMENT OF DIABETES MELLITUS\*

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THE purpose of these experiments was to study the effect of a mineral water when used for long periods in the management of diabetes mellitus. Recently companies bottling many of the highly advertised mineral waters have made extravagant claims for their water of beneficial effects in diabetes mellitus. With this in view the following investigation was undertaken to determine whether or not there was any demonstrable beneficial effect derived from the use of one of these waters.

#### CASE REPORTS

CASE 1.—R. P., male, aged thirty-nine years, first had symptoms of diabetes mellitus at the age of twenty-nine. The disease had run a rapid course, and he had, to the date of the experiments, lived on a quantitative diet with insulin for eight years. He was an intelligent, trained, and experienced patient. His usual diet contained carbohydrate, 105 gm.; protein, 127 gm.; and fat, 183 gm. With this diet he remained in good health but not free of morning glycosuria on an insulin dosage of 75 units.

CASE 2.—H. E., male, aged fifty years, had become diabetic at the age of forty and had required insulin treatment for seven years. This patient was also a very intelligent, trained, and experienced diabetic patient. His customary diet contained carbohydrate, 94 gm.; protein, 74 gm.; fat, 181 gm.; and cal., 2,301, estimated "G,"† or available glucose, 155 gm.; on this diet the urine was usually sugar-free with an insulin dosage of 50 units, divided into 40 in the morning and 10 in the evening.

\*From the department of medicine of Rush Medical College of the University of Chicago and the Presbyterian Hospital.

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†The "G," or available glucose, was calculated by the formula, "G" = C + .58P + .10F.

CASE 3.—W. S., male, aged thirty-two years, had first developed symptoms of diabetes mellitus in 1919 at the age of twenty. He first had insulin management in 1923 and went into coma in 1924 at the age of twenty-five. He had been, for seven years, at the time of these experiments on a diet of "G," 175 gm.; cal., 2,275, and insulin, 35 units before breakfast and 16 units before the evening meal. He had remained in good health but not free of glycosuria in the morning and was also an intelligent, well-trained and experienced patient.

CASE 4.—H. D., male, aged forty-five years, had first become diabetic in 1920 at age of thirty-four. By very accurate quantitative management of the diet, he was kept alive until the advent of insulin in 1922 at which time this therapy was started. For nine years and at the time of these experiments, he had been taking 50 units of insulin per day, and he had been in good health but not free of morning glycosuria. His usual diet was "G," 180 gm.; cal., 2,250. He was also a well-trained, intelligent, and experienced patient.

CASE 5.—H. A., male, aged fifty-eight years, first developed diabetes mellitus at age of forty-five. In 1923, at age of fifty years, he was placed on a diet of carbohydrate, 100 gm.; protein, 67 gm.; fat, 156 gm.; "G," 155 gm.; and cal., 2,072, and started on insulin, 32 units before breakfast and 16 units before the evening meal. At the time of these experiments he had been on this program for eight years, had remained in good health; the urine usually was free of sugar. He also was a well-trained, an intelligent, and experienced patient.

All of these patients had been continuously under the observation of Dr. R. T. Woodyatt.

*The Mineral Water.*—The mineral spring water used in these experiments was crystal clear and, according to the analysis submitted by the bottler, contained 120 parts per million of solid material. The distribution of the identified anions and cations was reported as follows:

|                                |    |                                |   |       |                   |
|--------------------------------|----|--------------------------------|---|-------|-------------------|
| H <sub>2</sub> CO <sub>3</sub> | as | CaCO <sub>3</sub>              | = | 38.00 | parts per million |
| Chlorides                      | as | Cl                             | = | 8.00  | parts per million |
| Sulphates                      | as | SO <sub>4</sub>                | = | 18.55 | parts per million |
| Silicon                        | as | SiO <sub>2</sub>               | = | 3.25  | parts per million |
| Aluminum                       | as | Al <sub>2</sub> O <sub>3</sub> | = | 3.52  | parts per million |
| Iron                           | as | Fe <sub>2</sub> O <sub>3</sub> | = | 3.58  | parts per million |
| Calcium                        | as | Ca                             | = | 4.46  | parts per million |
| Magnesium                      | as | Mg                             | = | 1.13  | parts per million |
| Sodium and Potassium           | as | Na                             | = | 24.3  | parts per million |
| Manganese                      | as | Mn                             | = | 0.08  | parts per million |
| Ammonia                        | as | NH <sub>3</sub>                | = | 0.04  | parts per million |
| Nitrates                       | as | NO <sub>3</sub>                | = | 0.02  | parts per million |

The radioactivity of the emanation, when in equilibrium with its parent, from one gallon of this water was reported to be in the neighborhood of  $1.5 \times 10^{-10}$  millicuries.

#### EXPERIMENTS

*Methods of Study.*—Each patient was hospitalized during the periods of study and placed on a diet and dosage of insulin that would permit a slight glycosuria during the entire 24 hours without acidosis. The same insulin preparation was used during all of the experiments. Two control periods were run on each patient with a week intervening. At the end of the second period the patient was discharged from the hospital using his usual diet, dose of insulin, and drinking one gallon of the mineral water each day. After using the water from one to seven months he was again hospitalized, placed on the

diet and dosage of insulin used in the control periods, the water continued, and the urinary glucose determined.

The glucose in the urine was determined by the Folin-Berglund method and polariscopy, the nitrogen by the Kjeldahl method and the creatinine by the picramic acid method. The latter was used for a check on the volume of urine.

*Experiment 1.*—Case of R. P., weight 73 kg. A diet of carbohydrate, 87 gm.; protein, 76 gm.; fat, 199 gm.; "G," 151 gm.; and cal., 2,443, with insulin, 32 units before breakfast, 16 units before the evening meal and 4 units at 3 A.M., permitted a glycosuria without acidosis. The average daily urinary glucose excretion during the first control period was 14.43 gm., and the urinary nitrogen, 11.1 gm. In the second control period the average daily urinary glucose excretion was 13.54 gm., and the urinary nitrogen was 12.8 gm. After he had drunk one gallon of the water per day for seven months, the average daily urinary glucose excretion was 15.76 gm., and the urinary nitrogen was 14.7 gm.

*Experiment 2.*—Case of H. E., weight 60 kg. A diet of carbohydrate, 92 gm.; protein, 54 gm.; fat, 172 gm.; "G," 141 gm.; and cal., 2,132, and twenty-four units of insulin before breakfast and 12 units before the evening meal permitted a continuous glycosuria without acidosis. The average daily urinary glucose during the first control period was 8.61 gm., and the nitrogen, 8.8 gm. In the second control period the average daily urinary glucose was 11.98 gm., and the nitrogen, 10.1 gm. After using one gallon of the water per day for one month, the glucose was 13.14 gm. and the nitrogen, 8.8 gm. After using the water for four months, the average daily urinary glucose was 12.50 gm. and the nitrogen was 10.1 gm. After using the water for seven months, the average daily urinary glucose excretion was 10.34 gm. and the nitrogen, 11.6 gm. In this experiment another control period was run after the water had been discontinued for one month. The urinary glucose then was 7.94 gm. and the nitrogen, 9.8 gm.

*Experiment 3.*—Case of W. S., weight 66 kg. A diet of carbohydrate, 92 gm.; protein, 79 gm.; fat, 187 gm.; "G," 156 gm.; cal., 2,367; and insulin, 28 units before breakfast and 12 units before the evening meal, permitted a slight continuous glycosuria without acidosis. The average daily urinary glucose excretion in the first control period was 9.11 gm. and the nitrogen, 10.5 gm. In the second control period the glucose excretion was 10.01 gm. and the nitrogen, 11.5 gm. After using one gallon of the water per day for three months, the average daily urinary glucose excretion was 8.51 gm. and the nitrogen, 12.4 gm. After using the water for six months there had been no reduction in the daily insulin requirements, and the experiment was discontinued without another period of study in the hospital.

*Experiment 4.*—Case of H. D., weight 65 kg. A diet of carbohydrate, 92 gm.; protein, 79 gm.; fat, 187 gm.; "G," 156 gm.; cal., 2,367; and insulin, 24 units before breakfast and 16 units before the evening meal, permitted a slight continuous glycosuria without acidosis. The average daily urinary glucose excretion during the first control period was 6.84 gm. and the nitrogen, 11.6

gm. During the second control period the glucose was 7.17 gm. and the nitrogen, 12.7 gm. After drinking one gallon of the water per day for three and one-half months, there had been no reduction in the daily insulin requirements, and the experiment was discontinued without another period of study in the hospital.

*Experiment 5.*—Case of H. A., weight 65 kg. A diet of carbohydrate, 92 gm.; protein, 79 gm.; fat, 187 gm.; "G," 156 gm.; cal., 2,367, and insulin, 32 units before breakfast and 16 units before the evening meal, permitted a continuous glycosuria without acidosis. The average daily urinary glucose excretion during the first control period was 12.99 gm. and the nitrogen, 14.7 gm. During the second control period the glucose excretion was 8.17 gm. and the nitrogen, 14.7 gm. After drinking one gallon of the water per day for seven months, there had been no reduction in the daily insulin requirements, and the experiment was discontinued without further study in the hospital.

#### SUMMARY

A study of the effect on diabetic patients of their drinking one gallon of mineral water per day for periods of from one to seven months was made. No beneficial effects could be demonstrated.

The author wishes to thank Dr. R. T. Woodyatt for his valuable advice and assistance during this study.

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## THE DYNAMIC CONSEQUENCES OF AN AURICULOPERICARDIAL FISTULA\*

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IT IS generally known that pericardial tamponade has a deleterious action on the circulation, and when acute and severe enough may lead to death. Animal experiments, particularly those of Katz and Gauchat,<sup>1</sup> have shown that the action of pericardial tamponade is primarily to interfere with the flow of blood into the intrapericardial veins. Intrapericardial pressures in excess of 300 mm. of oil (saline) lead to circulatory death. It is the fear of establishing fistula with the resulting tamponade effects that has acted as a deterrent to pericardial and cardiac punctures. However, there seems to be an impression that a communication between the pericardium and an auricle is less serious than a communication between the pericardium and a ventricle, the aorta or the pulmonary artery.

In the present investigation an attempt was made to determine the dynamic consequences of producing an auriculopericardial fistula, and in particular to see what risk, if any, was involved in such a procedure.

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\*From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital. Received for publication, October 30, 1933. Aided by the Frederick K. Babson Fund of the Michael Reese Hospital.

## METHOD

Six dogs, anesthetized with morphine and barbital, were used in this investigation. Artificial respiration was introduced and the chest opened. Chicago blue (15 mg. per kg.) was injected intravenously to render the blood noncoagulable. A pericardial cannula was inserted and connected with a saline manometer. The carotid artery, jugular vein, and a pulmonary vein branch were isolated and cannulated. The first was connected with a mercury U-tube manometer and the other two with saline manometers. After a control record was obtained on a smoked paper kymograph a small opening was made in the pericardium through which a rent about 5 mm. in length was made in the tip of the right or left auricle and the pericardial opening quickly closed. The changes in pressure during and following this procedure were recorded on the kymograph and the data analyzed. As a control 50 c.c. of blood were removed from an animal and quickly injected into the pericardium, and its effects studied.

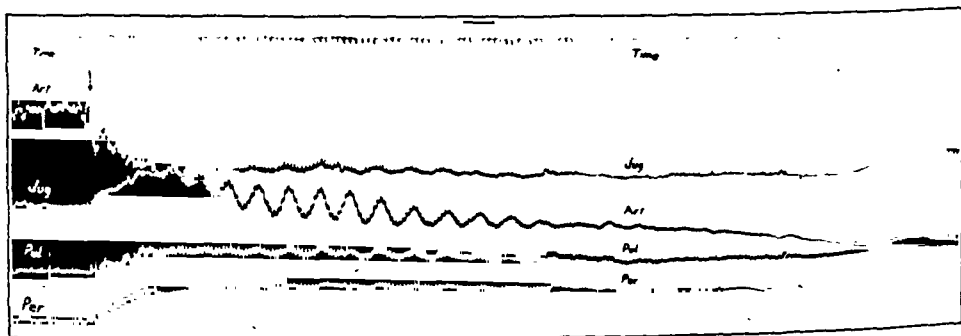


Fig. 1.—A graph showing the typical effect of an auriculopericardial fistula. Art, is the mean arterial pressure; Jug., Pul., and Per., represent the mean pressure, respectively, in the jugular vein, pulmonary vein, and pericardial sac. Time in five-second intervals. Arrow indicates time of establishment of auriculopericardial fistula. Note the Traube-Hering waves and the homologues in the venous and pericardial pressure curves.

## RESULTS

The results are summarized in Table I and a typical experiment shown in Fig. 1. After the establishment of the auriculopericardial fistula, there was an immediate drop in the mean arterial blood pressure and a corresponding rise in the mean pressures in the jugular vein, pulmonary vein, and in the pericardial sac, following which the pressures tended to level off but continued to change gradually in the original directions until death ensued. The mean arterial pressure just before death approximated 5 to 15 mm. of mercury. At this time the pulmonic venous pressure was found to exceed the jugular pressure in all but one experiment, regardless of whether the fistula was in the right or left auricle. The intrapericardial pressure in no instance rose above 140 mm. of saline following the establishment of the fistula, a figure which is about one-half of that found necessary to cause death with experimental pericardial effusion (Katz and Gauchat<sup>1</sup>). In some experiments the intrapericardial pressure was higher than the intravenous pressure; in others it was lower.



## DISCUSSION

The lack of correspondence of the intrapericardial pressure with the intravenous pressures is in part due to the inability to establish absolutely the correct zero pericardial pressure level. In addition, the cyclic heart action affects the pressure in the pericardial sac in an opposite manner to the extrapericardial venous pressures. For example, ventricular systole lowers the intrapericardial pressure, but raises the venous pressures. In some experiments the last factor may explain the presence of an intrapericardial pressure lower than the venous pressures. The presence of an intrapericardial pressure higher than the venous pressures in other experiments is more difficult to explain. It is conceivable, however, that with a rent in the auricle this part of the wall of the heart may act as a one-way flapper valve, preventing a flow back from the pericardial sac. In this way the pericardial sac might be considered as acting as a maximum pressure manometer.

TABLE I

| EX-<br>PER. | MEAN ARTERIAL<br>BLOOD PRESSURE |                 | MEAN PRESSURE<br>IN JUGULAR VEIN |                     | MEAN PRESSURE<br>IN PULMONARY VEIN |                     | MEAN<br>PRESSURE<br>IN PERI-<br>CARDIAL<br>SAC<br>BEFORE<br>DEATH | WHICH<br>AURICLE<br>CUT | TIME OF<br>DEATH<br>AFTER<br>AURICLE<br>CUT |
|-------------|---------------------------------|-----------------|----------------------------------|---------------------|------------------------------------|---------------------|-------------------------------------------------------------------|-------------------------|---------------------------------------------|
|             | CONTROL                         | BEFORE<br>DEATH | CONTROL                          | BEFORE<br>DEATH     | CONTROL                            | BEFORE<br>DEATH     |                                                                   |                         |                                             |
|             | mm Hg                           | mm Hg           | mm.H <sub>2</sub> O              | mm.H <sub>2</sub> O | mm.H <sub>2</sub> O                | mm.H <sub>2</sub> O | mm.H <sub>2</sub> O                                               |                         | min.                                        |
| 1           | 145                             | 5               | 25                               | 90                  | 65                                 | 110                 | 140                                                               | right                   | 13½                                         |
| 2           | 135                             | 5               | —                                | —                   | 60                                 | 120                 | 100                                                               | left                    | 8                                           |
| 3           | 55                              | 5               | 35                               | 90                  | 50                                 | 90                  | 140                                                               | right                   | 8½                                          |
| 4           | 60                              | 5               | 35                               | 50                  | 60                                 | 60                  | 55                                                                | right                   | 8½                                          |
| 5           | 90                              | 5               | 30                               | 90                  | 60                                 | 80                  | 70                                                                | left                    | 16                                          |
| 6           | 105                             | 15              | 60                               | 135                 | 100                                | 140                 | 120                                                               | right                   | 12                                          |

Tamponade per se cannot be responsible for the serious dynamic consequences of auriculopericardial fistula. Previous workers<sup>1</sup> have found that the pressure in the pericardium causing death in tamponade per se is decidedly greater than the pressure found in the pericardium at the time of death in our animals. This difference cannot be due to the loss of blood, which never exceeded 25 or 30 c.c. in our experiments. Furthermore, the removal of 50 c.c. of blood from the circulation and its rapid injection into the pericardial sac had no appreciable dynamic effect.

The other factor operating in auriculopericardial fistula appears to be the action of the fistula itself, and the following explanation is offered: When the fistula is made, the pressure in the auricle and pericardial sac tend to be equalized, the intrapericardial pressure rises and thereby increases the resistance to onward flow of blood from the extrapericardial veins. This in turn results in a damming up of blood in the extrapericardial veins with a consequent rise in their pressure. The rise in pressure, however, soon reflects itself in a similar elevation in the intrapericardial veins, auricle, and then in the pericardial sac itself. Thus a vicious cycle is started. There is no chance for

<sup>1</sup>Katz, L. N., and Gauchat, H. W.: Observations on Pulsus Paradoxus (With Special Reference to Pericardial Effusions). II Experimental. Arch. Int. Med. 23 371, 1924.

a gradient of pressure to be established between the intrapericardial veins and auricles (and the diastolic pressure in the ventricles) and the pericardial sac, which is essential to prevent stagnation of the circulation and death.

These experiments show that a persistent opening between an auricle and the pericardial sac is associated with danger, even though the amount of bleeding into the pericardial sac be small.

#### SUMMARY

The dynamic effects of a persistent auriculopericardial fistula experimentally produced were studied. It was found that the fistula resulted in a serious handicap to the normal circulation and quickly led to death. This effect is apparently not due entirely to a tamponade action per se but to a decrease in the pressure gradient between the extrapericardial veins and the ventricle during ventricular filling produced by the presence of the fistula.

We wish to thank Dr. Louis N. Katz, at whose suggestion this study was undertaken, for his advice and criticism.

### A TYPHOID-LIKE INFECTION ASSOCIATED WITH AN ORGANISM RESEMBLING *BACILLUS PROTEUS PSEUDOVALERIAE* (DE ASSIS)\*

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THE case here reported is one of infection with an organism closely resembling *Bacillus proteus pseudovaleriei* (de Assis).

The patient was a man of Canadian birth, a salesman by occupation, aged fifty-eight. The family and history were of no special interest except for the fact that four years prior to the present illness the patient developed hematuria and was operated upon for a papillomatous growth of the bladder. The growth was removed by fulguration in a one-stage operation, and radium seeds were implanted in the base of the growth. Recovery was uneventful, but several months later the patient developed difficulty in urination and was able to urinate only while lying down. It became necessary to remove a stone which had formed around one of the radium seeds and which acted as a ball valve. This procedure was accompanied by severe cystoscopic shock and rigors. During the time that the patient suffered from urinary symptoms prior to operation, he had a considerable number of mild attacks of precordial pain on exertion that had been diagnosed clinically as well as by electrocardiogram as of coronary origin. These symptoms ceased after the operation.

The present illness followed a late lunch which consisted of a spiced beef sandwich. At the time this was eaten the patient thought it had a peculiar taste. He experienced a sense of epigastric distress that became worse the next day. However, on the following day he dined in a speakeasy and had two gin cocktails before dinner. The second day he had a sense of dull epigastric ache. On the third day he had severe chills lasting several hours followed by fever and sweating. During the next two weeks the symptoms became more severe and the chills and fever more frequent. There was no regular interval between the attacks.

\*From the Department of Pathology and the Fourth Medical Service of Bellevue Hospital. Received for publication, November 9, 1933.

Anorexia and prostration were marked, and the patient lost weight rapidly and entered into a typhoid state. At this point he was admitted to the Fourth Medical Service at Bellevue Hospital. At the time of admission abdominal pain on the right side was the predominant symptom. Examination of the abdomen was negative.

Shortly after admission the patient became jaundiced. The van den Bergh test was positive and the stools were clay colored. There was rigidity in the right upper quadrant and some tenderness in the region of the gallbladder. The blood chemistry was negative. The patient began to have steady hiccoughing, and the urine was found to contain pus. Several infusions of glucose were given intravenously. The blood chemistry now showed nonprotein nitrogen 75, sugar 120, and creatinine 2.5. Blood cultures taken shortly after admission were positive and a gram-negative bacillus was found in the blood. The Widal reaction was negative. The same organism was isolated from the feces. The temperature curve gradually subsided and after a stay of three months in the hospital the patient went to his home. Following his return to New York in October he complained of severe pains in the muscles of the upper thorax. These pains were so severe as to require the use of a sedative. The x-ray of the chest and spine were negative, and the patient was given a series of heat and ultraviolet ray treatments followed by complete cessation of the pains. At the present time he is in good health and has regained weight and strength.

During bacteriologic studies of blood cultures from this patient, we isolated a bacillus which appears to be closely similar to the *Proteus pseudovaleriei* isolated by A. de Assis in Brazil in 1926.<sup>1</sup>

Blood cultures were made in vitamin broth and nutrient agar pour plates. The first culture, taken April 29, 1932, the day after admission, showed a gram-negative motile bacillus in both broth and plates after twenty-four hours. Another culture was taken May 7, eight days later, which was also positive. A third culture, taken May 26, was sterile.

Agglutination tests: Normal saline suspensions of the growth on an eighteen-hour agar slant and an eighteen-hour broth culture were tested with dilutions of 1:50 and 1:100 specific typhoid and paratyphoid A and B sera. No agglutination occurred.

Repeated Widal tests of the patient's serum proved negative with typhoid and Para A and Para B.

On May 26, nineteen days after this organism had been recovered from the patient's blood, the patient's serum was tested with the organism. Serum agglutinated through 1:2,500, negative 1:5,000. On repeating the test complete agglutination was found in 1:3,200, negative 1:5,000. Tested with typhoid Para A and Para B the serum again proved negative.

Stool culture of June 16, seventeen days after defervescence, on Endo plates showed a single colony which resembled morphologically and culturally, the organism isolated from the blood, and agglutinated with the patient's serum of May 26 through 1:3,200, negative 1:5,000.

These results showed that the organisms isolated from the blood and from the stool were identical, and the fact that it agglutinated with the patient's serum in a dilution of 1:3,200 showed that it was the cause of the infection.

In 1910, Rottkay<sup>2</sup> described a fatal case of typhoid-like disease from which he was able to recover an organism related to *B. Proteus vulgaris* (Hauser) which he isolated from the feces and after death from the spleen and ileum.

Boycott<sup>3</sup> in 1906 isolated from the stool of a case of atypical typhoid, two strains of Valerei which did not agglutinate with the patient's serum. This patient's serum was agglutinated with Para B. although no paratyphoid organisms were isolated from the stools.

De Assis in 1926 recovered an organism from the blood of a case of typhoid-like infection on two occasions, that strongly agglutinated with the patient's serum, but did not agglutinate with typhoid, Para A and Para B. He called his organism *Proteus pseudovaleriei*.

## DESCRIPTION

Morphology: a gram-negative actively motile bacillus of varied size, from coccoid to the longer threadlike forms, morphologically resembling the typhoid bacillus.

Subcultures were made on the following media:

- a. Nutrient agar slant showed a moist spreading grayish white fluorescent growth.
- b. Broth showed a rapid growth, of even turbidity, with the formation of a collar or ring at the surface, and sediment after forty-eight hours. No pellicle was formed.
- c. Blood pour plates showed greenish colonies similar to typhoid.
- d. Endo plates showed bluish transparent colonies similar to typhoid. Broth cultures kept in the ice box a week or more and subcultured on endo showed presence of "phage plaques."
- e. Triple sugar agar slants were inoculated with colonies from Endo plates and showed acid "butt" with gas production and a colorless slant similar to the paratyphoid-enteriditis group.
- f. Litmus milk showed acid in twenty-four hours but was not clotted. Later it became decolorized, with partial coagulation. No digestion or peptonization.

These cultures were transplanted to various carbohydrate fermentation broth tubes containing Andrade as the indicator. Dextrose, levulose, maltose, mannite, saccharose, xylose, salicin, arabinose, raffinose, and glycerol were fermented with gas production. Lactose was not touched until after seven days, then it gave a faint acid reaction. Dextrin showed slight reaction after seventy-two hours. No gas was produced in either lactose or dextrin. Inulin and dulcitate were not touched. These fermentation tests were repeated twice and all three reactions on the same lot of media proved the same. From 50 per cent to 100 per cent gas was formed.

## PATHOGENICITY

We inoculated two 250 gm. guinea pigs intraperitoneally with 0.5 and 0.75 c.c. of eighteen-hour-broth culture. After three hours the pigs became quiet and drowsy, did not eat, and the hair looked roughened. Eighteen hours later they showed signs of prostration, had not eaten any food, and when placed on their backs were barely able to right themselves. Within twenty-four hours both pigs were dead. The same organism was recovered in pure culture from the peritoneum and heart's blood of both pigs.

There was a considerable amount of injection at the site of the inoculation. The peritoneum contained about 10 c.c. of mucoid fluid. The mesentery and omentum were injected. The suprarenal glands were hemorrhagic. The spleen of one pig was slightly enlarged, the gallbladder was quite distended.

## DISCUSSION

The biologic characteristics of our organism places it in the genus *Proteus*. In 1906 Boycott described a bacillus (*valeriei* 21) which our organism closely

resembles in motility and fermentation, although our bacillus does not ferment dextrin and dulcitol; while his does not ferment salicin, and does not touch lactose at all. De Assis, in 1926, described a bacillus which our bacillus resembles, except that ours ferments saccharose, raffinose, and glycerol, while his did not. Our bacillus resembles Castellani's *Proteus asiaticus* in fermentation, but differs in its reaction on litmus milk, and in motility. Our strain resembles Hauser's vulgaris in its reaction on saccharose, dextrin, dulcitol, and glycerol, but it does not resemble it in its reaction on arabinose, salicin, raffinose, mannitol, or litmus milk. The difference in the reaction on dextrin may be due to the variation of dextrin.

Our organism resembles the *Proteus pseudovaleriei* of de Assis in its fermentation reactions more closely than it does Hauser's Vulgaris or the Valerei of Castellani or of Boycott, and by the fact that his patient, like ours, recovered, while all of the others died.

#### SUMMARY

An organism isolated from two different samples of blood and from the stool of a patient with a typhoid-like infection, strongly agglutinated with the patient's serum but did not agglutinate with typhoid, Para A or Para B. This organism may be classed with that of de Assis.

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# LABORATORY METHODS

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## AN IMPROVISED BACTERIOLOGIC INCUBATOR\*

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A HIGH-PRICED bacteriologic incubator costing from \$100.00 to \$300.00 can be improvised at the very moderate cost of \$20.00 or less with no sacrifice in the quantity or quality of the results to be obtained from its use. The improvised incubator can be assembled in less than fifteen minutes with only four articles that can be easily and quickly obtained, especially for those who live in a city.

A small, used refrigerator made to hold 25 pounds of ice makes a very satisfactory sized cabinet for an incubator large enough to do the bacteriologic work of a moderately large hospital. I assembled an excellent incubator, using a top icer of the above size which was purchased from a storage company for \$1.50. The refrigerator was as good as new and the factory finish on it was still good.

The second article needed for the construction of the incubator is an electric heating element and regulator or electric thermostat. This can be purchased from any good laboratory supply company for not more than \$16.00 and large enough to keep the entire inside of the refrigerator at 37° C. or body heat.

The third article needed to make the first class incubator is an accurate thermometer with which to regulate the electric thermostat and check the temperature of the incubator when in use. The thermometer can be obtained from the same supply company as the thermostat for a dollar or less or a suitable one can be obtained at a drug store.

The fourth article needed is a one-holed stopper which can be obtained from the supply company.

With these articles at hand one is ready to begin assembling the incubator. First open the door to the refrigerator and remove the tube connecting the ice compartment with the storage compartment by raising up and pulling out on the bottom of the tube. This leaves an opening down through the bottom of the refrigerator. Next, the thermostat is placed on the lower shelf in the left rear corner and the electric cord will just be in position to go down through the hole in the lower shelf, but first the electric connection will need to be temporarily removed as the hole in the floor of the refrigerator is not large enough to pass the connection through. After the cord has been

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\*From the Hankey Laboratory.  
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put through the floor, the electric connection can be replaced and made ready to plug into a wall socket supplying 110 volt current.

If a laboratory type of thermometer is to be used, an inch hole should be drilled through the top door and a cork with a hole made through it the size of the thermometer can be inserted in the hole. The thermometer is then suspended with the bulb well within the refrigerator by passing it through the stopper so that the mark for body heat or 98.6° F. is well above the outside of the cabinet. Any accurate thermometer may be used by placing it on the shelf inside the refrigerator and the readings taken quickly upon opening the door or before the thermometer has time to change enough to be of any importance. However, for greater accuracy the laboratory thermometer through the pierced wall is recommended, so that the temperature can be read without opening the door.

For the proper growth of aerobic organisms a half-inch hole should be drilled through the top near the center for a small amount of ventilation to maintain the oxygen supply even during prolonged incubations.

You are now ready to start the regulation of the thermostat for the desired temperature by plugging into the 110 volt current and closing the doors. Keep a frequent watch of the rising temperature and as soon as the desired temperature is attained take notice if the heater bulb is still lighted up, by observing through the ventilator opening in a darkened room or by opening the door slightly. If it is still lighted up open the door and turn the knulled knob of the regulator on the thermostat to the left until the light goes out. Then watch the temperature again. If it should go too low the next time turn the regulator to the right but let sufficient time elapse to insure even distribution of the heat before making any changes in regulation. Let the incubation continue for some time after the right temperature is attained to make sure that an exact adjustment has been made. You have now completed a very efficient and serviceable incubator.

If it is desired to use the incubator on 32 volt current, purchase the thermostat without the heater bulb and replace same with a 100 watt 32 volt bulb and regulate as above.

# DETERMINATION OF THE $P_H$ OF NORMAL AND MALIGNANT TISSUES WITH THE GLASS ELECTRODE AND VACUUM TUBE NULL INDICATOR\*

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IN A previous communication<sup>1</sup> the authors have discussed the results of  $P_H$  measurements with the glass electrode on certain normal and tumor tissues. It is the purpose of this paper to describe the method. The apparatus used requires comparatively little electrical shielding and is simple, stable and semiportable in nature.

## METHOD

The method for measurement of the potentials developed by the glass electrode cell was the conventional Poggendorf circuit with the exception that a vacuum tube null instrument was used. A Leeds and Northrup type K potentiometer was used to furnish the known opposing potential.

## THE NULL INDICATOR

The present circuit differs from those previously described by the authors<sup>2, 3, 4</sup> mainly in that the bias and free grid-adjusting resistors are in parallel with the power supplies instead of in series. This arrangement gives equal stability with improved sensitivity and simplicity of operation.

Fig. 1 with the list of circuit constants fully explains the electrical values.

## OPERATION

Potentiometer  $P$  is adjusted to furnish correct opposing potentials in the usual manner.

To adjust the null apparatus, batteries  $A$  and  $C$  are connected and allowed to operate for twenty minutes or a half hour in order that the various chemical and thermal equilibria may be established.

With no connection at jack  $S$ ,  $Rg$  is set so that galvanometer  $G$  gives no deflection when tapping switch  $T$  is pressed. Plug  $B$  is now inserted in  $S$ , and  $Rb$  is adjusted until  $G$  again gives no deflection when  $T$  is depressed.  $B$  is removed from  $S$  and replaced by  $E$  which completes the external cell-measuring circuit.  $P$  is adjusted until  $G$  again gives no movement, and the E.M.F. of cell  $HR$  is read from  $P$ .

## SHIELDING

Under ordinary conditions when glass electrodes with resistances of less than 15 megohms are used, no shielding of the external circuit is required.

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As indicated in the list of constants the tubes  $VV$  are shielded electrostatically (and to a slight extent, electromagnetically) to aid in setting  $R_g$  when the grid circuit is open. The general rule for shielding is obviously that when the electrical strays reach a magnitude comparable with the currents flowing in the input measuring circuit, screening is necessary. A connection to ground is sometimes helpful but for the present work was unnecessary.

# SENSITIVITY, STABILITY, LEAKAGE

With the circuit described which includes a portable galvanometer, a sensitivity of from 2,500 to 4,000 mm. per volt is obtained. In other words, when the bridge is out of balance by an amount representing 0.01  $P_H$  a deflection of from 1 to 2 mm. is noted on galvanometer  $G$ . Tube characteristics and resistance conditions in the input circuit cause considerable variation in this factor.

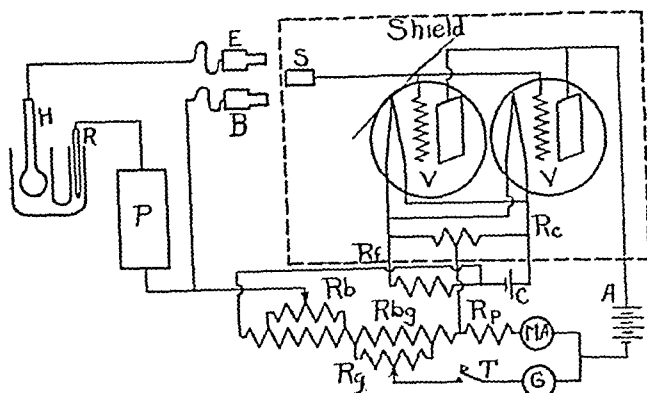


Fig. 1.—Schematic circuit for glass electrode E. M. F. measurements. Circuit constants. *Shield*, Metallic enclosure for tubes to assist in adjusting circuit when the grid is "free." *H*, Haber type glass electrode. Corning Glass Works' 015 glass. Resistance 5-12 megohms. Diameter of bulb approximately 8 mm. *R*, *Ba*. . . . . Conventional method for securing known potential. . . . . small radio type. *S*, Jack to fit *E* and *B*. *V*, *V*, "Duovac" 1.1 volt 270 . . . . . triodes. *A*, Battery of 12 large size flashlight cells, approximately 18 v. . . . . midtapped resistance. *Rf*, Filament resistor 1.9 ohms. . . . . 2-volt storage cell of approximately 50 ampere-hour capacity. *Rbg*, . . . . . tor equipped with four movable contacts. *Rb*, *Rg*, Variable resistors of . . . . . type, 500 ohms each. *Rp*, Plate resistor, 3,000 ohms. *MA*, Plate circ . . . . . 0-1 or 0-1.5. Not necessary for actual operation but helpful in mal . . . . . Portable pointer type galvanometer, resistance 1,100 ohms; sensitivity 8 mm. per microampere. *T*, Tapping switch.

Mechanically, the apparatus is stable, and for ordinary conditions no cushioned mounting is necessary. Electrically, the drift is very small when the various thermal and chemical equilibriums have been reached—often less than the equivalent of a millivolt per hour. The room temperature, of course, must remain constant since the power supplies have temperature coefficients. That this is a major factor in the drift characteristic was demonstrated by allowing the room temperature to change slowly and taking time-room-temperature and time-drift relationships. The curves obtained were similar in shape with the drift change following the thermometer change with a delay of from twelve to fifteen minutes.

Leakage difficulty is negligible if care is taken to have the connection between  $H$  and  $E$  short and well insulated. For the latter purpose, dry wooden

supports have always been found adequate. Results with the equipment when used on a constant system are reproducible to a few hundredths of a millivolt.

### ELECTRODES

Haber type electrodes *H* made from Corning 015 glass were used having diameters of approximately 8 mm., and resistances ranging from 5 to 12 megohms. Some of the electrodes were filled with a Clark and Lubs buffer ( $P_H$  about 2) saturated with quinhydrone. Others were filled with  $N/10$  HCl (approximately) saturated with quinhydrone.<sup>6</sup>

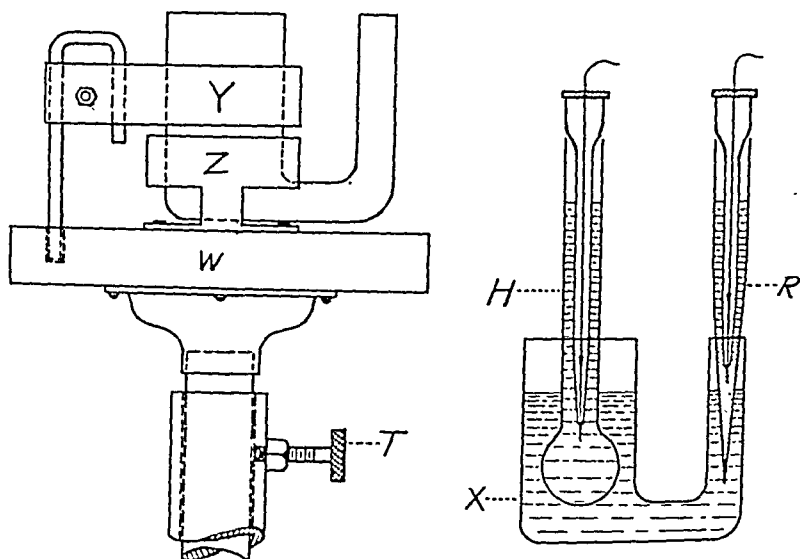


Fig. 2.—Electrode assembly shown in detail. *H* is the glass electrode (see circuit constants for Fig. 1) containing buffer or HCl saturated with quinhydrone; *R* is a calomel Ball type reference electrode. The electrical contact to each electrode is made by a fine platinum wire which is sealed into a glass capillary. A copper wire soldered to each platinum wire makes contact with the measuring device. The electrode vessel *X* is fixed rigidly to the wooden base *W* by the collar *Y* and socket *Z*, both of spring brass. Electrodes *R* and *H* are clamped in position above *X* and contacts with the material to be measured are secured by raising the assembly *WXYZ*. The latter is held in place by thumb screw *T*.

The complete electrode assembly is shown in Fig. 2.

The reference electrode *R* was the general type described by Ball.<sup>8</sup>

The complete electrode combination was as follows:

|    |                   |                         |       |                            |               |    |
|----|-------------------|-------------------------|-------|----------------------------|---------------|----|
| Pt | Normal<br>Calomel | Buffers<br>or<br>Tissue | Glass | $N/10$ HCl<br>or<br>Buffer | - Quinhydrone | Pt |
|----|-------------------|-------------------------|-------|----------------------------|---------------|----|

### CALIBRATION OF GLASS ELECTRODE

The glass electrode was calibrated before and after each determination against Clark and Lubs buffers.<sup>9</sup> The values used for calibration were 6.55, 6.98, 7.14, 7.35, 7.53, and 7.76. These were compared by both the hydrogen and quinhydrone electrodes.

We observed, as did Britton and Robinson,<sup>7</sup> that the glass electrode within this  $P_H$  range adheres to its calibration potentials for several hours. These observers found their electrodes in this region to come to equilibrium almost instantaneously, whereas, in our work about four minutes were required before a steady potential was attained. This can hardly be attributed to time to dissipate a charge; for after equilibrium was attained, the glass electrode was removed from solution, then replaced and a reading again taken which was always found to be the same as that at equilibrium.

The electrode was cleaned by washing with distilled water of the same temperature as the electrode. It was dried by carefully wiping with jagged edges of torn filter paper.

### $P_H$ OF TISSUES

Different normal and cancer tissues from rats and mice were used. The animal was killed, the tissue immediately removed, quickly macerated and

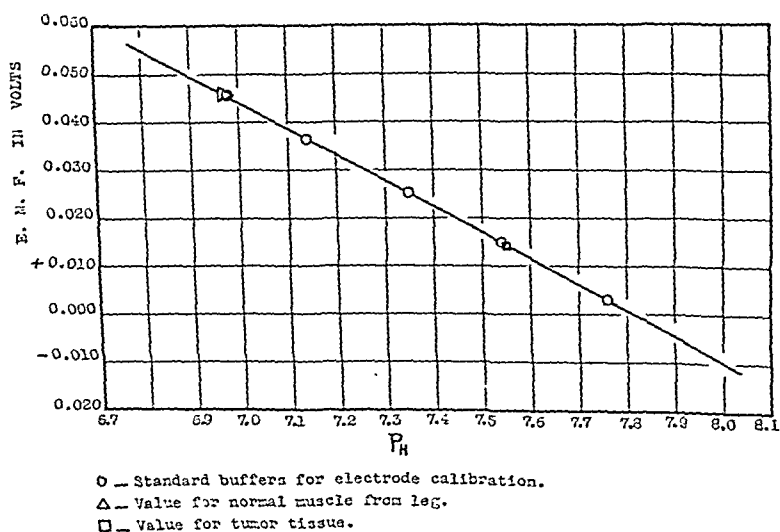


Fig. 3.—Mouse with Tumor 63.

placed in electrode vessel (Fig. 2) and forced into the connecting arm so that contact could be made with the reference electrode  $R$ . As soon as the glass electrode bulb was completely covered with tissue, a thin layer of paraffin oil was added and E.M.F. measurements taken. The system came to equilibrium within three or four minutes and remained constant for approximately another three minutes, after which there was the usual slow drift toward a lower  $P_H$ . The  $P_H$  values of the tissues were obtained directly from the calibration curve (see Fig. 3). With the apparatus in readiness the  $P_H$  measurements were completed within eight minutes after the death of the animal.

In Tables I, II, and III are some results obtained by the method outlined.

TABLE I  
NORMAL RATS

| NUMBER  | $P_H$<br>MUSCLE FROM LEG | $P_H$<br>LIVER |
|---------|--------------------------|----------------|
| 1       | 6.99                     | 6.78           |
| 2       | 6.89                     | 6.80           |
| 3       | 6.88                     | 6.98           |
| 4       | 6.72                     | 6.88           |
| 5       | 6.70                     | 6.84           |
| Average | 6.84                     | 6.86           |

TABLE II  
TUMOR RATS

| NUMBER  | $P_H$<br>FLEXNER-JOBLING<br>RAT CARCINOMA | $P_H$<br>NORMAL MUSCLE<br>FROM LEG | $P_H$<br>LIVER |
|---------|-------------------------------------------|------------------------------------|----------------|
| 1       | 7.34                                      | 6.83                               | 7.05           |
| 2       | 7.39                                      | 6.87                               | 6.93           |
| 3       | 7.41                                      | 6.92                               | 6.95           |
| 4       | 7.29                                      | —                                  | 6.87           |
| Average | 7.36                                      | 6.87                               | 6.95           |

TABLE III  
MICE WITH TUMOR 63

| NUMBER  | TUMOR TISSUE | NORMAL MUSCLE FROM LEG |
|---------|--------------|------------------------|
| 1       | 7.54         | 6.97                   |
| 2       | 7.39         | 6.78                   |
| 3       | 7.24         | 6.81                   |
| Average | 7.39         | 6.85                   |

Furusawa and Kerridge,<sup>10</sup> and Carlström, Ege, and Henriques<sup>11</sup> obtained average  $P_H$  values of 6.89 and 6.88, respectively, for tissues from leg muscles of normal rats. Our results for similar normal tissues are therefore in close agreement.

#### SUMMARY

A method has been described for the determination of  $P_H$  in normal and malignant tissues by means of the glass electrode. The electrical apparatus is comparatively simple, stable and semiportable. For the normal animal tissues compared, our results are in close agreement with other workers.

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## THE RECORDING ELECTRODYNAMIC BRAKE BICYCLE ERGOMETER\*

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WE HAVE elsewhere briefly described a bicycle ergometer which has since been rebuilt, further improved, and modified.<sup>1</sup> It fulfills the need for a work machine which not only provides a satisfactory brake and accurately measures work done, but which simultaneously records the constancy of the output and the speed of working. These are factors of importance in physiologic studies of exercise so far as the cost of muscular work is related to the speed and steadiness with which it is performed. The constancy of the output is a variable hitherto unmeasured by the ergometers in common use. The chief purpose of this communication is to present the circuit diagram of the new recording electrodynamic brake ergometer and to describe its method of calibration.

*Principle.*—The principle of the electrodynamic brake resides in the fact that a separately excited direct current generator is the load. When a generator armature is rotated and the field is excited, a voltage is generated. If the armature is then connected to a resistor, a current will flow. This current reacting against the field magnetism develops a counter torque or braking effect. The work done in unit time is the torque multiplied by the distance it moves, or the rate of work in watts is the generated voltage times the current flowing.

*Description of the Apparatus.*—As constructed, the ergometer consists of a standard woman's bicycle frame from which the wheels and handlebar have been removed, mounted on a portable supporting structure along with the necessary accessories. These consist of a generator, storage battery, load resistor, field control resistor, ammeter, voltmeter and electrical revolution counter. The set-up is illustrated in Figs. 1 and 2.

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The generator is a 12 volt Dodge automobile starter. The series winding is disconnected and the shunt winding is connected for separate excitation. The generator is directly driven from the main sprocket which has 100 teeth. There are 10 teeth on the generator sprocket, giving a generator to pedal speed ratio of 10. A fly wheel is affixed to the generator shaft to assist in the maintenance of constant speed.

The load resistor is of advance wire which has a resistance practically independent of temperature. The resistor is connected across the armature

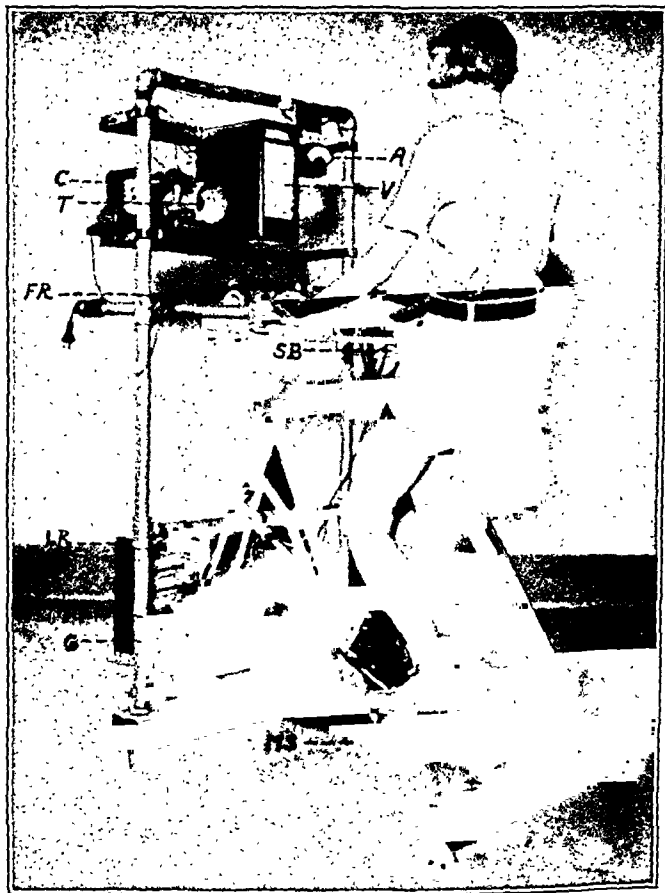


Fig. 1.—Photograph of the recording electrodynamic brake bicycle ergometer. *A*, ammeter; *V*, recording voltmeter; *T*, timer; *C*, electric clock; *FR*, field control resistor; *SB*, switch board; *LR*, load resistor; *F*, flywheel; *G*, generator; *MS*, main sprocket. The rotating switch for the electrical revolution counter may be seen just above the hub of the main sprocket. The storage battery and charger are located to the right of the generator and the flywheel.

with a switch  $S_3$  which may be opened for testing purposes. It is made up of 6 coils of wire in parallel so that the value of the load may be changed for subjects of widely different strengths.

The voltmeter is a 15 volt graphic recording instrument. The clock is driven by a Warren self-starting synchronous motor, and has chart speeds of 3 inches per minute and 3 inches per hour. It is started and stopped by switch  $S_4$  which is mounted within easy reach of the subject. The lever that

changes the chart speed may also be reached by the subject. The recording needle is large and easily visible, so that the meter is distant reading at all times and may be made recording at will.

A 12 volt storage battery is used to supply the current for the field excitation, the revolution counter and the timer. For convenience a portable charger is mounted on the platform which holds the battery, and it may be charged during long riding bouts. The circuits are so arranged that the battery may also be used to furnish power to operate the generator as a motor for calibration purposes.

The ammeter is a 15 ampere distant reading instrument. It indicates the value of the excitation current when the ergometer is in use and the value of

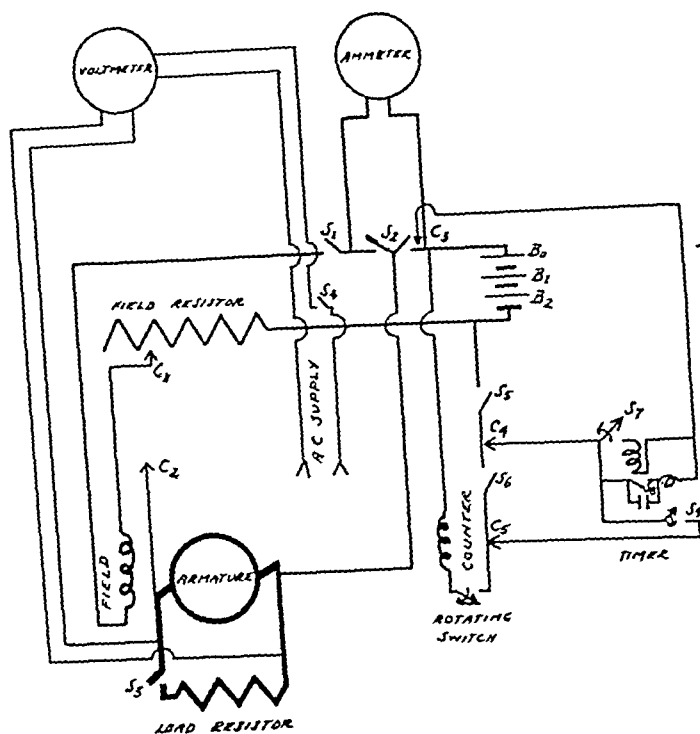


Fig. 2.—Circuit diagram of the electrodynamic ergometer.

the current to the armature for motor action during calibration procedures. Field currents from 1 to 7 amperes are those normally used. Switch  $S_1$  is in the field circuit. The double throw switch  $S_2$  is used to send the armature motor current direct to the armature, or through the ammeter in parallel with the field current. The ammeter may thus be made to indicate either the field current alone, or the combined field and motor armature currents.

The field current is controlled by a 20 inch, 12 ampere, 8.5 ohm resistor with a sliding contact,  $C_1$ . This field resistor is mounted in front of and close to the operator, so that it may be reached and controlled while riding. The moving contact,  $C_2$ , may be connected to various points on the battery for motor operation purposes.

The revolution counter is operated by an electromagnet. A rotating switch on the main sprocket makes contact once each revolution. With switch  $S_6$  closed,  $S_5$  starts and stops the counter. It may be automatically kept in and out of service by a timing device parallel with  $S_6$ . A three conductor cable connects the timer with the ergometer. The contacts are made as indicated by  $C_3$ ,  $C_1$ , and  $C_5$ . When  $S_6$  is open, the timer is in control of the counter, but when  $S_6$  is closed, the timer is inoperative. With  $S_5$  open, both the counter and the timer are out of service.

In the timer itself,  $S_7$  is a switch operated by an electric clock and closed every fifteen seconds. The current through  $S_7$  operates a relay that closes  $S_5$ . The current through  $S_8$  operates an electromagnet that rotates a drum  $\frac{1}{100}$

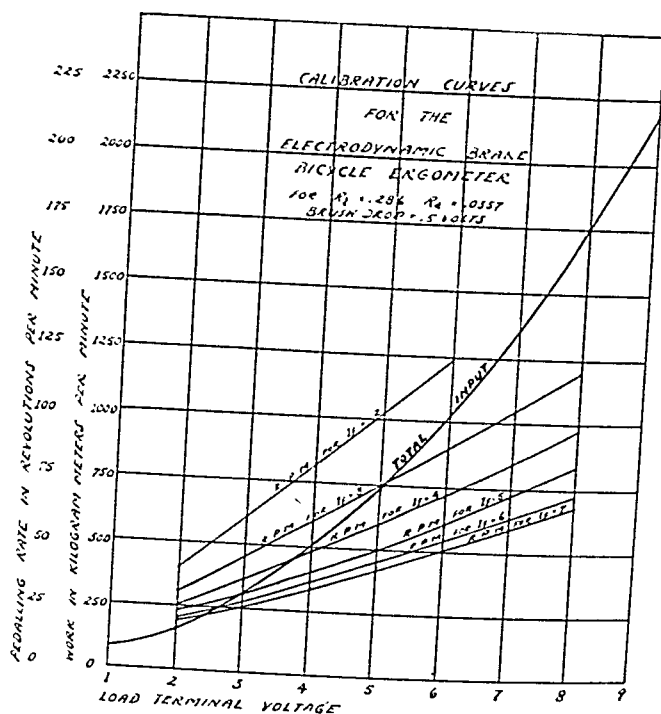


Fig. 3.—Curves for the conversion of load terminal voltage into rate of working and for the estimation of pedalling rate under various field current and load terminal voltage conditions.

of a revolution. The switch  $S_8$  is paralleled by a condenser to cut down sparking. The contact on the drum,  $S_9$ , is opened and closed for different lengths of time depending upon the calibration of insulating strips on its surface.

**Calibration of the Ergometer.**—The output of the subject is the input to the ergometer. This input is composed of the stray power losses and the armature generated power. For any speed and field current and no armature current, a certain amount of power is required to run the ergometer. This power is assumed to be independent of armature current and is called the stray power loss. The armature developed power is the product of the armature current and the generated voltage. It is possible to determine the value of these losses for any load terminal voltage. Their sum plotted against load



terminal voltage is the calibration curve of the instrument. This is presented in Fig. 3, the speed vs. load terminal voltage curves being plotted on the same sheet.

The stray power losses are determined by making a series of runs, varying the speed but keeping the field current constant for each. The speed, field current, armature voltage, and armature current are recorded. The stray power losses are then calculated and the corresponding load terminal voltage determined. The stray power losses for the various speeds and field currents are then spotted on a graph and a composite curve drawn which gives the loss for any voltage.

The armature developed power is calculated in the following way: The generated voltage is the sum of the load terminal voltage, the brush drop, and the resistance drop of the armature proper and the leads. The armature current is the load terminal voltage divided by the load resistance. The load resistance is constant and is independent of temperature. The brush drop is assumed constant at 0.5 volt. The resistances of the armature and leads vary with the temperature, but since they are small, the error due to temperature change is small.

The resistance of the armature, the leads and the load resistor was determined, employing the volt ampere method, and using currents over the range that would be obtained under load conditions. The brush drop was estimated by plotting a series of curves of no load terminal voltage vs. speed, for constant field current. These curves were projected back to the zero speed line, their intersections with this line being taken as the brush drop. In making the no load runs the generator was operated as a motor. Switch  $S_2$  was opened,  $S_2$  was closed up,  $S_1$  was closed, the field current  $I_f$  was adjusted to some value, and contact  $C_2$  was connected to some point on the battery, as  $B_1$ . The apparatus now operates motor action at no load, and the input to the armature is the stray power losses plus a small loss due to brush drop and armature resistance. The field current was again adjusted and the values  $B$ ,  $E_t$ ,  $I_f$ , and R.P.M. were recorded.  $S_2$  was closed down. The armature and field currents both pass through the ammeter. They were recorded as  $I_{(a + f)}$ .  $S_2$  was closed up and a new contact made on the battery, and the data were obtained over the range of speeds to be used. Then the entire procedure was repeated for all values of field current over the range to be used.

The stray power loss is the generated voltage times the armature current. The generated voltage is related to the no load terminal voltage by the following equation:  $E_g = E_t + (0.5 + I_a R_a)$ .

The no load data for the stray power calculations were arranged as follows:

|   |       |       |               |       |        |      |            |
|---|-------|-------|---------------|-------|--------|------|------------|
| B | $E_t$ | $I_f$ | $I_{(a + f)}$ | $I_a$ | R.P.M. | S.P. | $E_{load}$ |
|---|-------|-------|---------------|-------|--------|------|------------|

where  $B$  is the connection on the battery,  $E_t$  is the no load terminal voltage,  $I_f$  is the field current,  $I_{(a + f)}$  is the sum of the field and armature currents,  $I_a$  is the no load armature current, R.P.M. is revolutions per minute, S.P. is stray power loss, and  $E_{load}$  is the load terminal voltage for a generated voltage of the same value as the generated voltage for the corresponding  $E_t$ .

For any stray power loss the generated voltage is calculated as before. The load terminal voltage is related to the generated voltage by the following equation:

$$E_g - 0.5 = E_t (1 + \frac{R_a}{R_{load}})$$

The armature developed power is the product of the armature load current and load generated voltage. This is expressed in terms of load terminal voltage by the following equation:

$$W = \frac{E_t^2}{R_{load}} (R_L + R_a) + \frac{0.5 E_t}{R_{load}}$$

The watts are changed to Kg.m./min. by the following equation:

$$\text{Kg.m./min.} = W(6.15075).$$

*Criticism of the Instrument.*—The possible sources of error are, (a) stray power losses, (b) armature  $I^2R$  losses, and (c) brush drop loss. These are all small and their possible variation from the mean is also small. Their total effect is assumed to be less than errors due to the inability of the subject to keep the voltage constant, and may therefore be neglected.

The validity of this instrument has not been determined. It purports to measure the output of mechanical work performed by human subjects. The comparison of the results obtained with some outside criterion is difficult to effect, and further, is unnecessary because the instrument is built upon a brake principle of proved characteristics already demonstrated and long used with success in engineering.

The degree to which the measuring devices for the determination of mechanical work, constancy, and speed give invariable readings under identical conditions is high. For a given field current and pedalling rate, the load terminal voltage is always the same, irrespective of the environmental conditions and the experience of the operator. Constancy and speed are measured by synchronized, electrically driven clocks. Under identical field currents and load terminal voltages, the speed of pedalling may be well duplicated, both during protracted bouts of exercise and during pieces of work performed on different days. For example, at a given field current an experienced rider holds the voltmeter needle at the prescribed point with such accuracy that the speed of pedalling, estimated for one minute at five-minute intervals during a one-hour period of exercise, will not vary by more than one revolution per minute plus or minus, and will remain at the modal R.P.M. 83 per cent of the time.

We believe this measuring device is especially good because of its objectivity and administrative economy. The recording voltmeter graphs the constancy of the performance and automatically records the data necessary for the calculation of the rate of working. The speed of pedalling is also automatically counted at any desired intervals during the exercise. The apparatus is movable and may be used by a subject without the assistance of an operator.

*Principles for the Selection of Load and Speed Conditions.*—Table I presents the rate of working and the speed of pedalling under a wide range of

field current and load terminal voltage variations. The exercises performed under these loads and speeds have been classified physiologically as to their severity. In Table II this classification is presented along with the data for performing the different exercises in various gradations on the electrodynamic brake bicycle ergometer.

TABLE I

THE RATE OF WORKING AND SPEED OF PEDALLING UNDER VARIOUS CONDITIONS OF FIELD CURRENT AND LOAD TERMINAL VOLTAGE\*

| LOAD TERMINAL VOLTAGE | RATE OF WORKING<br>Kg.m./Min. | SPEED OF PEDALLING AT VARIOUS FIELD CURRENTS |        |        |        |        |        |
|-----------------------|-------------------------------|----------------------------------------------|--------|--------|--------|--------|--------|
|                       |                               | 2 AMP.                                       | 3 AMP. | 4 AMP. | 5 AMP. | 6 AMP. | 7 AMP. |
|                       |                               | R.P.M.                                       | R.P.M. | R.P.M. | R.P.M. | R.P.M. | R.P.M. |
| 2                     | 167                           | 40                                           | 32     | 26     | 23     | 20     | 19     |
| 3                     | 309                           | 60                                           | 45     | 36     | 32     | 28     | 25     |
| 4                     | 501                           | 78                                           | 59     | 48     | 41     | 38     | 34     |
| 5                     | 740                           | 104                                          | 73     | 58     | 50     | 47     | 43     |
| 6                     | 1,031                         | 124                                          | 88     | 72     | 60     | 55     | 51     |
| 7                     | 1,366                         |                                              | 104    | 84     | 74     | 64     | 60     |
| 8                     | 1,749                         |                                              | 120    | 98     | 84     | 72     | 68     |

\*For a load resistance of 0.286.

TABLE II

SPEED AND LOAD CONDITIONS FOR GRADATIONS OF THE VARIOUS TYPES OF EXERCISE

| RATE OF WORKING SPEED OF PEDALLING FIELD CURRENT LOAD TERMINAL VOLTAGE |        |         |       |
|------------------------------------------------------------------------|--------|---------|-------|
| Mild Exercise                                                          |        |         |       |
| Kg.m./min.                                                             | R.P.M. | Amperes | Volts |
| 167                                                                    | 40     | 2       | 2     |
| 167                                                                    | 32     | 3       | 2     |
| 309                                                                    | 45     | 3       | 3     |
| 309                                                                    | 36     | 4       | 3     |
| Moderate Exercise                                                      |        |         |       |
| 309                                                                    | 60     | 2       | 3     |
| 309                                                                    | 45     | 3       | 3     |
| 501                                                                    | 78     | 2       | 4     |
| 501                                                                    | 59     | 3       | 4     |
| 740                                                                    | 73     | 3       | 5     |
| 501                                                                    | 48     | 4       | 4     |
| Violent Exercise                                                       |        |         |       |
| 740                                                                    | 104    | 2       | 5     |
| 1031                                                                   | 88     | 3       | 6     |
| 1031                                                                   | 124    | 2       | 6     |
| 1366                                                                   | 104    | 3       | 6     |
| 1366                                                                   | 84     | 4       | 7     |
| 1749                                                                   | 120    | 3       | 7     |
| 1749                                                                   | 84     | 5       | 8     |
| 1749                                                                   | 98     | 4       | 8     |
| Exhaustive Exercise                                                    |        |         |       |
| 740                                                                    | 73     | 3       | 5     |
| 1031                                                                   | 88     | 3       | 6     |
| 1031                                                                   | 72     | 4       | 6     |
| 1366                                                                   | 84     | 4       | 6     |
| 1366                                                                   | 74     | 5       | 7     |
| 1749                                                                   | 84     | 5       | 7     |
| 1749                                                                   | 72     | 6       | 8     |

The exact load and speed conditions to be selected depend upon the age and the development of the individual subject. The child does not possess the muscular strength to perform exercises at the upper ranges of field cur-

rent. Nor can the upper ranges of speed be well borne. The young adult is better suited than the subject in any other age group to carry on violent exercises of speed. He can be expected to perform creditably under the low field current and high voltage conditions associated with the highest pedalling rates. Muscular power is at its height during middle age and the exhaustive exercises of endurance at high voltage and field currents are then well tolerated. Table II is based upon the performance of strong, healthy, young adult women in training for physical education. At a given rate of working, they invariably prefer an exercise with a low field current and high speed of pedalling to one with a high field current and a slower pedalling rate. Exercises of speed are performed well, but these subjects do not possess the muscular power to pedal against a strong resistance. The limitations of the table are obvious. Mild exercise for one individual may be moderate for another. Under certain speed and load conditions an exercise may be easily borne for ten minutes, but carried on for an hour, it becomes exhaustive.

*Operation of the Ergometer.*—Having decided upon the field current and the load terminal voltage with the aid of the tables, close switch  $S_3$  and switch  $S_1$ , leaving  $C_2$  disconnected. Adjust the field current to the proper value by moving the slide contact on the field resistor. The proper selector on the timing device is connected at  $S_6$  and the drum is rotated until it has at least one movement to make before  $S_2$  contacts. The roll mechanism of the voltmeter is started by closing the clock motor control switch  $S_1$ . The revolution counter is read, pedalling the bicycle is begun, and  $S_2$  is closed. The subject now has fifteen seconds or more to get to stable conditions before  $S_2$  contacts and the revolution counter begins to operate. The counter will operate for the chosen time and will then be automatically disconnected. The reading of the counter is then recorded, and the subject continues to ride and record to the end of the run.

*Comment on the Uses of the Instrument.*—The ergometer has thus far been used for the following four types of problems: (1) The repetition of identical work under constant physiologic conditions for the study of training. (2) The repetition of identical work under variable physiologic conditions for the study of their effect on its cost. (3) The study of the physiologic effect of varying speed at a constant mechanical output. (4) The graphic record of fatigue curves. The advantages of the recording ergometer for repetitious work are obvious. The instrument lends itself especially well to studies necessitating variations in the speed of pedalling at constant output. A single manipulation, the alteration of the field current, accomplishes this since the rate of working remains constant as long as the load terminal voltage is unchanged.

#### SUMMARY

The electrodynamic brake principle has been applied to a stationary bicycle ergometer. It has furnished an easily adjustable brake with a constant coefficient of friction. The introduction of a recording voltmeter has made it possible to measure with accuracy the rate and steadiness of working. The

instrument may be used under a variety of speed and load conditions without the assistance of an operator.

The ergometer was built by J. S. Hipple, mechanician, in the shops of the Medical School. We wish to acknowledge his helpful suggestions and cooperative assistance in the development of this project.

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## THE TRYPTOPHANE CONTENT OF BLOOD SERUM\*

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### A NEW TECHNIC

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ARTHUR T. BRICE, JR., B.A., M.T., PALO ALTO, CALIF.

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MITCHEL and Hamilton<sup>1</sup> in their work on the amino acids quote J. M. Looney, as follows: "The multiplicity of the methods proposed for the estimation of tryptophane and the inconsistency of the values given are sufficient evidence of the importance attached to the subject and the worthlessness of most of the methods." Such a situation offers little encouragement to present another tryptophane method, but it does suggest that conflicting results in differentiating between normal and pathologic material might be avoided by some standardization of procedure. The tryosine content of the plasma proteins has been shown to be constant, and the determination of this amino acid can, therefore, be used as a measure of the total protein. Tryptophane, however, in the light of our present knowledge occupies a position more characteristic of an independent variable. Glycocol, alanine, serine, cysteine, aspartic acid, and glutamic acid are among the glucose formers of the amino acids. Valeine, leucine, isoleucine, phenylalanine and tyrosine give rise to beta-hydroxybutyric acid. Histidine and tryptophane are exceptions. It has been shown that tryptophane forms neither glucose nor beta-hydroxybutric acid in the organism of the dog. The tryptophane content of the plasma proteins has not been accurately established, and the practical application of a blood serum tryptophane method does not, therefore, at the moment seem apparent. The lack of such a present application, however, should not deter us from keeping our faculties of observation alive and seeking to learn what we can of physiologic or pathologic significance about this unique amino acid. It is with the hope that it may be used for such a purpose that the following technic for blood serum tryptophane is suggested.

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\*From the U. S. Veterans' Administration Hospital, Palo Alto, California.  
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The specificity of the Adamciewicz reaction for tryptophane is entirely beyond question, and as applied in the Hopkins and Cole Glyoxylic test it has been shown to be quantitative within wide limits. It is particularly suitable for clinical laboratory application, as it is simple and rapid. I have developed its application to human blood serum and prepared an artificial color standard which is practically permanent if handled with ordinary care. The use of such a standard is open to criticism from the standpoint of absolute values, but aside from its comparatively negligible expense it has a distinct advantage over the chemical standard, which is notably unstable as a means for the accumulation of data. The chemical standard being subject to deterioration an element of error would likely creep into any series for which it might be employed, whereas with the artificial standard, assurance is had that the basis of comparison will not change throughout the series.

#### STANDARD

The standard is prepared from stock 0.05 per cent aqueous solutions preserved with a crystal of thymol of the DuPont water soluble dyes violamine blue and pontamine fast blue. Exactly 2.8 c.c. of each of these two stock solutions is diluted to 100 c.c. with a solution consisting of 3 per cent gelatin, 0.9 per cent sodium chloride, 5 per cent oxalic acid, and several crystals of thymol dissolved by heating on a boiling water-bath, cooling, and clearing by sedimentation, centrifugation, or filtration. A good grade of gelatin must be used and the diluting solution come through crystal clear with a trace of amber color. The DuPont dyes, which were developed specifically for use as color standards, are of absolutely uniform color value. They are light fast over a period of years if the solutions are kept in dark bottles. The gelatin vehicle is used to provide in the standard a molecular density, approximating that of the unknown blood serum mixtures. This standard may be prepared in the laboratory or may be purchased ready for use from the Hynson, Westcott and Dunning Company of Baltimore, Md., to which firm I am indebted for acquainting me with the properties and use of the DuPont water soluble dyes.

#### REAGENTS

*Hopkins-Cole Glyoxylic Reagent.*—To one liter of a saturated solution of oxalic acid add 60 gm. of 2 per cent sodium amalgam and allow the mixture to stand until the evolution of gas ceases. Filter and dilute with 2 or 3 volumes of water.

The glyoxylic reagent prepared according to Benedict's formula gives practically identical values with the above, but I have found it not to keep as well and old reagents to give less brilliance of color.

*Sulphuric Acid 95 per cent.*—A chemically pure or reagent quality of concentrated acid must be used, the usual U. S. P. grades giving cloudy solutions.

#### TECHNIC

a. Clear blood serum is diluted 1/10 with distilled water and 1 c.c., equivalent to 0.1 c.c. of serum, placed in a clean glass test tube.

b. Add 1 c.c. of glyoxylic reagent and then 3 c.c. of sulphuric acid, a few drops at a time, while shaking the tube.

c. Let stand for several minutes for the maximum color to develop and the mixture to cool somewhat and then compare in the colorimeter with the standard set at 15 or 20.

$$\text{Calculation: } \frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 100 = \frac{\text{mg. of total tryptophane per}}{\text{cent of blood serum}}$$

The standard will be found to be slightly more brilliant photometrically than most of the blood specimens but to provide a satisfactory basis for comparisons. The unknown solutions being strongly acid and quite hot must be handled carefully.

TABLE I  
RECOVERY OF ADDED TRYPTOPHANE

| SPEC. NO.                 | ORIGINAL SPEC.<br>IN MG. PER<br>CENT | TRYPTOPHANE<br>ADDED IN MG.<br>PER CENT | TRYPTOPHANE<br>RECOVERED IN MG.<br>PER CENT | PER CENT<br>RECOVERED |
|---------------------------|--------------------------------------|-----------------------------------------|---------------------------------------------|-----------------------|
| 1                         | 167.0                                | 100.0                                   | 250.0                                       | 83                    |
| 2                         | 181.8                                | 100.0                                   | 285.7                                       | 104                   |
| 3                         | 148.1                                | 100.0                                   | 259.7                                       | 112                   |
| 4                         | 190.4                                | 100.0                                   | 285.7                                       | 95                    |
| 5                         | 166.7                                | 50.0                                    | 212.9                                       | 92                    |
| 6                         | 200.0                                | 50.0                                    | 250.0                                       | 100                   |
| 7                         | 153.8                                | 50.0                                    | 200.0                                       | 92                    |
| 8                         | 166.7                                | 50.0                                    | 210.5                                       | 98                    |
| 9                         | 178.5                                | 25.0                                    | 200.0                                       | 86                    |
| 10                        | 190.0                                | 25.0                                    | 210.5                                       | 80                    |
| 11                        | 181.8                                | 25.0                                    | 206.1                                       | 97                    |
| 12                        | 153.8                                | 25.0                                    | 181.8                                       | 112                   |
| Average per cent recovery |                                      |                                         |                                             | 96                    |

The appended table reports some recoveries of pure tryptophane added to sera in the diluting water (Table I).

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2. Hawk, P. B., and Bergheim, O.: Practical Physiological Chemistry, ed. 9, Philadelphia, 1926, P. Blakiston's Son & Co., p. 125.

## A COMBINATION CONDENSING AND RECEIVING VESSEL\*

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DURING the past few months the authors have had occasion to make a number of protein analyses. Since only small amounts of material were available, Cavett's<sup>1</sup> micromodification of the Van Slyke method was used. In the determination of arginine, an aliquot of the basic fraction is hydrolyzed with strong NaOH, which converts arginine into ornithin and urea. After hydrolysis, ammonia is distilled into standard acid. In the original method of Holm<sup>2</sup> the ammonia is distilled through a condenser, but in Cavett's modifica-

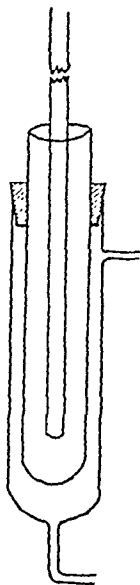


Fig. 1.

tion, it is distilled into a receiving tube containing standard acid. A large test tube is usually employed as a receiver and must always be placed in a beaker of ice in order for it to function as a condenser. Although this method gives satisfactory results, it is not convenient, and necessitates a supply of ice. Consequently, a combined condensing and receiving vessel was constructed which functions better and which uses circulating water as the cooling agent. The construction of the vessel is shown in Fig. 1.

The condenser shell was constructed from glass tubing 5 cm. in diameter and 22 cm. in length. A water inlet was placed at the bottom of the tube and

\*From the Department of Agricultural Chemistry, University of Arizona.  
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an outlet was placed 4 cm. from the top of the tube. The receiving vessel was a pyrex test tube 3 cm. in diameter and 20 cm. in length. A rubber stopper (size No. 9) was bored to receive the test tube.

The standard acid is placed in the tube and the distillation conducted in the usual way. A current of tap water cools the test tube, which serves as a condenser. When the distillation is complete, the test tube may be removed and the solution titrated.

The authors have secured satisfactory results with this apparatus. It has been used for the determination of arginine in protein hydrolysates, and in the microdetermination of ammonia in biologic fluids and in bacterial cultures. It could also be used in micro-Kjeldahl determinations, eliminating a special condenser.

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2. Holm, G. E.: A Modification of the Apparatus for the Determination of Arginine Nitrogen by Van Slyke's Method, *J. Am. Chem. Soc.* 42: 611, 1920.

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#### DOG-OPERATING TABLE\*

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SEVERAL months ago I was confronted with the task of outfitting a laboratory for dog surgery. A definite problem was encountered in connection with securing suitable dog-operating tables. After some preliminary experimentation with the various types of tables with which I was familiar, all of which proved to present disadvantages, it was decided to attempt to design a more satisfactory type of apparatus. The design of the dog-operating table which was finally developed and which experience at the Louisiana State University Medical Center has shown to be particularly useful and convenient, is herewith submitted in the belief that it embodies features which may be of interest to others working in the educational and laboratory fields.

The characteristics which were stressed in designing the table were: (1) So far as possible, an all metal construction; (2) portability, such that the table could be readily handled by one person; (3) quick and easy disassembly of the entire apparatus; (4) absence of all pockets and depressions in which dirt might accumulate; (5) accessibility of all parts for easy cleaning with soap and water; (6) a wide range of utility; and (7) sturdiness and stability.

The table is constructed of pipe iron, angle iron, and monel metal, the only wood used in its construction being two monel metal covered boards which constitute a troughlike support for the animal's body.

\*From the Department of Surgery, Louisiana State University Medical Center, and the Charity Hospital.  
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*Dimensions:* Fig. 1 shows the completely assembled table. The over-all dimensions of this table, exclusive of the instrument tray (which is removable) are as follows: height 40 inches, length 47 inches, width  $16\frac{1}{2}$  inches. Fig. 2 is a photograph of the bare frame of the table. For the most part this frame consists of pipe iron, of 1 inch diameter, outside measure, the pieces being cut to length and solidly welded together. The over-all dimensions of the frame of the table (exclusive of the instrument tray) are: height  $36\frac{1}{2}$  inches, length

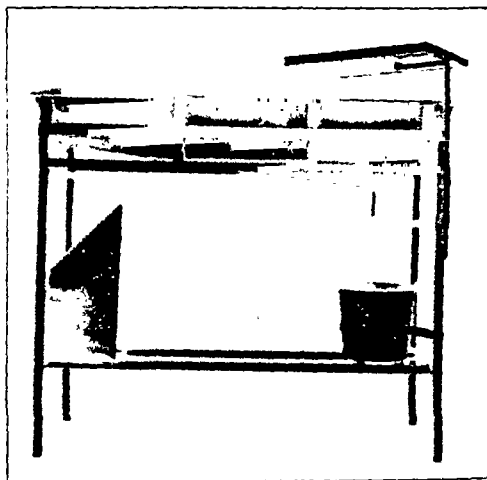


Fig. 1.

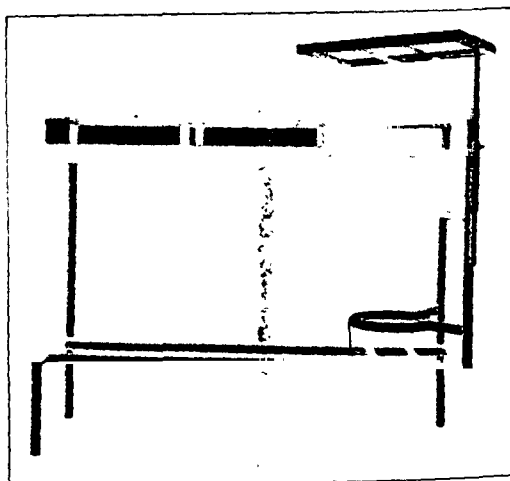


Fig. 2.

46 inches, width 16 inches. The extreme top of the frame is made of angle iron which measures  $1\frac{1}{4}$  inches on a side. The angle iron portion of the frame is open at the extreme right end, for the insertion of a monel metal pan, subsequently to be described, which slides in and out on the lower surface of the angle iron, like a drawer. The cross braces of pipe iron at the bottom of the table are ten inches on center from the floor. The end cross braces of pipe iron at the top of the table are respectively  $25\frac{1}{2}$  inches (right end of the table),

and  $31\frac{1}{2}$  inches (left end of the table), on center from the floor. The side braces of pipe iron at the top of the table are  $32\frac{1}{2}$  inches on center from the floor. At the right end of the table, above the cross braces nearest the floor, there is an arrangement made of strap steel, for the reception of a white enamel ware bucket. This consists of a ring suitably braced both to the legs of the table, and also to the base on which the enamel ware bucket rests. The base for the bucket is provided by two cross pieces of strap steel, welded to the lower cross braces of the table. The steel used in this part of the construction is 1 inch wide, and  $\frac{1}{8}$  inch thick. All joints are firmly welded.

The instrument tray measures  $17\frac{1}{2}$  by 13 inches. The frame of the tray consists of angle iron, welded at the corners in the form of a rectangle. The angle iron measures one inch on a side. Two cross braces of strap steel are welded across the bottom of the frame of the tray, and flush with its lower surface. They measure 1 inch in width and  $\frac{3}{16}$  inch in thickness. The tray

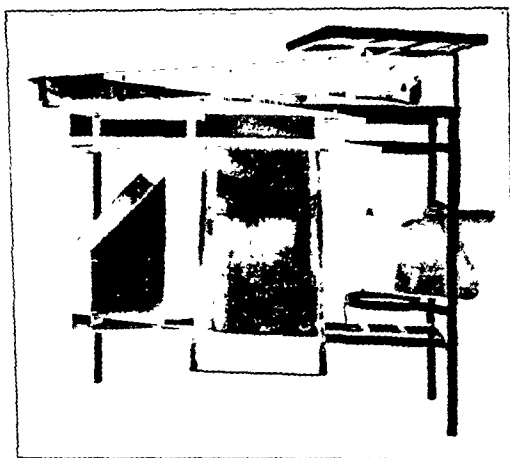


Fig. 3.

is supported by a right-angled steel rod. The horizontal limb of this rod measures 7 inches in length, and is welded both to the midportion of one end of the angle iron frame of the tray, and also to the midportion of the proximal strap-steel crossbar. The vertical limb of the rod measures 22 inches in length and  $\frac{9}{16}$  of an inch in diameter. The vertical limb of the rod provides support for the instrument tray in the following manner: A tubular piece of pipe iron, 6 inches in length is welded to one leg of the table by the intermediation of two offset pieces of strap steel, 1 inch in length and  $\frac{3}{16}$  of an inch in thickness. Through this piece of pipe iron, the vertical rod of the instrument tray passes in a snug fit. The tray is made adjustable for height by means of a collar and set screw, the position of which can be varied at will. The distance between the floor and the lowermost extremity of the offset pipe iron support for the instrument tray, is 26 inches. The instrument tray is not only adjustable for height, but swings freely on its supporting rod through an angle of 360 degrees.

Fig. 3 shows the frame of the table together with the accessory parts. These consist of (1) a monel metal instrument tray which fits inside of the instrument tray frame previously described, (2) a sheet monel metal drip pan, which slides in the groove provided by the angle iron top of the table, (3) a monel metal cabinet for the reception of ether cans, masks and other incidental paraphernalia connected with the anesthetization of the animal, and (4) a monel metal, wood, and strap-iron top constructed in a V-shape on which the animal lies.

The instrument tray pan is made to fit snugly into the angle iron tray frame, previously described. It measures slightly less than 17 inches in length, and slightly less than 13 inches in width, and is one inch deep. The edges are neatly turned over a heavy wire frame. The tray is neatly soldered so that it does not leak.

The drip pan is constructed of monel metal, and is of such dimensions that it provides a good sliding fit for the angle iron top of the table. Two flanges,  $\frac{7}{8}$  of an inch in width are provided on the edges at the top of the pan, one projecting from either side, on which the pan slides. The pan is  $1\frac{1}{2}$  inches deep at its shallower end, and 6 inches deep at its deepest part. The bottom of the pan slopes considerably at both ends, and also slightly on both sides. At the center of the extreme lower edge of the pan is soldered a drain spout which is  $2\frac{1}{2}$  inches in length, and  $\frac{3}{4}$  of an inch in diameter. This extreme lower edge of the pan is  $6\frac{1}{2}$  inches from the end of the table, and the drain spout centers accurately over the receptacle for the enamel ware bucket—or over the bucket itself when the bucket is in place. The upper edge of this drip pan is turned neatly over a heavy wire frame. The pan is neatly soldered so that it does not leak, and inasmuch as its sides slope in all directions, it drains very efficiently.

The cabinet for anesthetic paraphernalia is made of monel metal. It is  $12\frac{1}{2}$  inches wide, and 9 inches from front to back. The back of the cabinet is 18 inches in height. The sides of the cabinet at the top slope toward the front which is  $9\frac{1}{2}$  inches in height. The edges of the top of the cabinet are neatly turned over a heavy wire frame. The front of the cabinet projects for a distance of  $1\frac{1}{4}$  inches in the form of a flange at the sides, the dimensions of the front of the cabinet being  $14\frac{1}{2}$  inches in length, and  $9\frac{1}{2}$  inches in height. The flanges fit against the legs of the table, and prevent the cabinet from being displaced backward or inward. Riveted to the back of these projecting flanges, are a pair of strap metal hooks, one placed above the other, and one pair on either end. These hooks engage studs which project inward from the legs of the table, and prevent the cabinet from being accidentally displaced forward or outward. The cabinet is easily removable, however, by raising it straight up, thus disengaging the hooks from the studs. The cabinet rests on the cross supports of pipe iron at the bottom of the table. It is neatly soldered, so that it will not leak.

The top of the table on which the animal rests consists of a V-shaped trough, fashioned from two monel metal covered wooden boards. These boards measure 8 inches wide,  $47\frac{1}{2}$  inches long, and  $\frac{7}{8}$  of an inch in thickness.

As previously mentioned, the two boards form a trough, being separated throughout by a distance of  $1\frac{1}{2}$  inches. Four similar supports of angle iron bolted at regular intervals to the bottoms of the monel covered boards maintain the angle of the trough, and support this trough on the extreme edges of the top of the table frame. These angle iron supports are made of 1 by  $\frac{3}{16}$  inch cold rolled steel stock, bent on a template. They consist of a horizontal bar to which is welded at three places an inverted W-shaped piece of steel. The table top rests on the extreme upper edge of the angle iron frame of the table proper. The nature of the construction prevents displacement of the top of the table from side to side. Metal stops welded to the extreme end of the frame of the table proper, at its open end, prevent the top of the table from becoming displaced in that direction. The top of the table is, however, readily lifted, vertically.

All iron and steel parts of the table are finished with several coats of white enamel.

In operation, the table is extremely satisfactory. Not only is it pleasing to the eye, but all parts are easily removed without necessarily disturbing any other part of the table. The anesthetist's cabinet is disengaged by simply lifting it, and it can be emptied and cleaned with soap and water. The drip pan slides freely from the end of the table without disturbing the trough on which the dog lies. This pan is made entirely of rustless metal, and can be scrubbed into a condition of perfect cleanliness with soap and water. The instrument tray and instrument tray frame can be disassembled by a simple lifting movement. Similarly, the white enamel bucket, although it is entirely stable in its ring stand, can be readily lifted from its position and thoroughly cleaned. The top of the table, consisting of the trough in which the animal lies, although it is thoroughly stable, with respect to end and sidewise motion, can be readily lifted vertically, together with the animal if desired, without disturbing the rest of the ensemble.

NOTE.—This table was skillfully constructed jointly by the pipe-fitting, sheet metal working, carpenter, and paint shops of the Charity Hospital of New Orleans to my plans and specifications, and I am indebted to the mechanics for certain constructional modifications of the original design. The cost of the completely finished tables was \$39.60 apiece.

## ESTIMATION OF PLASMA BILIRUBIN<sup>2</sup>

### A COMPARATIVE STUDY OF THE VAN DEN BERGH AND THE THANNHAUSER AND ANDERSEN PROCEDURES

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THE purpose of this communication is to record a series of quantitative determinations of plasma bilirubin by the van den Bergh and the Thannhauser and Andersen techniques, and to compare the two methods *with respect to their applicability in clinical work*.

Van den Bergh<sup>1, 2, 3</sup> noted that with his procedure the protein precipitate, formed by the addition of alcohol to serum, may, at times, carry with it considerable amounts of bilirubin and, as a consequence, values obtained for serum bilirubin may be low. Such loss of bilirubin, however, did not occur or was negligible when sera contained no "direct" bilirubin. Thannhauser and Andersen<sup>4</sup> attempted to eliminate this source of error by coupling the bilirubin with the diazo reagent before precipitation of proteins with alcohol. Thus, according to the van den Bergh technic,<sup>2</sup> the serum (or plasma) proteins are first precipitated with alcohol and the protein-free portion is treated with the diazo reagent; whereas, with the Thannhauser and Andersen procedure<sup>4</sup> the diazo reagent is added to the serum (or plasma) *before* precipitation of the proteins. Thannhauser and Andersen also suggested the addition of ammonium sulphate to ensure complete precipitation of protein. Comparison of the values obtained by these two methods<sup>4, 6, 7, 8, 9</sup> indicates that loss of bilirubin by occlusion in the protein precipitate is considerably reduced by the Thannhauser and Andersen technic.

In this Clinic, the van den Bergh method has been used since 1922. Over five thousand determinations have been made, and correlation between plasma bilirubin values and clinical findings has been satisfactory, with one exception; in cases of subsiding jaundice, there appears to be no relationship between the color of the skin and the amount of bilirubin in the blood. This absence of relationship was thought to be due to the rate at which bilirubin is liberated from the tissues in this condition; at times, liberation of bilirubin from the tissues may be slow and the rate at which the pigment enters the blood may be much lower than the rate at which it can be removed. This would explain the frequent finding, in such cases, of jaundice of the skin in spite of normal or nearly normal amounts of bilirubin in the blood. In view of the definitely established fact that the van den Bergh procedure may not be quantitative when the blood contains "direct" bilirubin, it is necessary to seek an explanation of the generally satisfactory results with this technic in

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this Clinic. I was, therefore, prompted to compare the van den Bergh procedure with that of Thannhauser and Andersen and correlate the data with clinical findings.

#### METHODS

Oxalated plasma was used and all tests were commenced within one hour of collection of blood. Van den Bergh's cobalt-sulphate standard was used.<sup>6</sup> All determinations were carried out by the writer.

*Van den Bergh Technic.*—Two volumes of 95 per cent alcohol were well mixed with one volume of plasma and the mixture was centrifuged. To 1 c.c. of the clear supernatant fluid was added 0.5 c.c. of 95 per cent alcohol and 0.25 c.c. of freshly prepared diazo reagent. Fifteen minutes were allowed for full color development. The solution was then diluted with 66 per cent alcohol until the color matched that of the standard. In estimating the bilirubin, a dilution factor of 1 in 5 was used.<sup>10</sup>

*Thannhauser and Andersen Technic.*—There was 0.5 c.c. of freshly prepared diazo reagent mixed with 1 c.c. of plasma, and fifteen minutes allowed for full color development. There were 2.5 c.c. of 95 per cent alcohol and 1 c.c. of saturated aqueous solution of ammonium sulphate then added. After thorough mixing and centrifuging, a portion of the supernatant fluid was removed and diluted with 33 per cent alcohol until the color matched that of the standard. In estimating the bilirubin, a dilution factor of 1 in 4 was used.\*

#### DISCUSSION OF RESULTS

There is fairly general agreement that loss of bilirubin due to occlusion in the protein precipitate does not occur or is negligible when there is no "direct" bilirubin in the blood. In such cases, there are no significant differences between values obtained by the two methods. In this investigation, therefore, only plasmas containing "direct" bilirubin were used. All showed a "biphasic" direct reaction. In all, one hundred determinations were made in forty-three cases. The plasma bilirubin ranged between 0.3 and 32.0 units. Losses of bilirubin with the van den Bergh technic were calculated from the differences between values obtained by the two methods, it being assumed that no loss occurred with the Thannhauser and Andersen technic. The combined results are shown in Table I.

TABLE I  
SHOWING LOSS OF BILIRUBIN WITH THE VAN DEN BERGH METHOD

|                                      | BILIRUBIN<br>RANGE<br>(UNITS) | NUMBER<br>OF<br>TESTS | PERCENTAGE LOSS |     |       |       |       |
|--------------------------------------|-------------------------------|-----------------------|-----------------|-----|-------|-------|-------|
|                                      |                               |                       | NONE            | -20 | 21-40 | 41-60 | 61-80 |
| Combined data                        | 0.3-32.0                      | 100                   | 44              | 21  | 14    | 16    | 5     |
|                                      | - 4.0                         | 66                    | 40              | 8   | 4     | 10    | 4     |
|                                      | 4.1-32.0                      | 34                    | 4               | 13  | 10    | 6     | 1     |
| Subsiding jaundice                   | 1.0-13.2                      | 27                    | 0               | 1   | 5     | 16    | 5     |
| All cases, except subsiding jaundice | 0.3-32.0                      | 73                    | 44              | 20  | 9     | 0     | 0     |
|                                      | - 4.0                         | 51                    | 39              | 9   | 3     | 0     | 0     |
|                                      | 4.1-32.0                      | 22                    | 5               | 11  | 6     | 0     | 0     |

It will be observed that, with the van den Bergh technic, losses of bilirubin ranged between zero and over 60 per cent. When the data are grouped

\*In 65 of the 100 determinations, the protein precipitate and supernatant fluid as rubin values obtained by the assumed (1:1) the significance of the results.

calculated from the volume of The differences between bili- estimated factors were not sufficient to affect

according to concentration of bilirubin, it would appear that, in general, the higher the concentration the greater the loss. Losses ranging from zero to over 60 per cent were, however, still noted regardless of concentration. In view of the observation that the van den Bergh method was found unsatisfactory in cases of subsiding jaundice, it is of interest to note that the greatest losses occurred in this type of case; of 27 such determinations, losses exceeded 40 per cent in 21. In all other cases, the loss of bilirubin with the van den Bergh technic was relatively small; though losses were greater with plasmas of high, than with those of low, bilirubin content, they never exceeded 40 per cent and, in most, were less than 20 per cent regardless of concentration. That the marked losses of bilirubin noted in the cases of subsiding jaundice were not accidental is shown by repeated determinations in the same individual (Tables II, III, and IV).

TABLE II

SHOWING A SERIES OF DETERMINATIONS IN THE SAME INDIVIDUAL  
HOSPITAL No. 3122/33. DIAGNOSIS: CATARRHIAL JAUNDICE

| PLASMA BILIRUBIN<br>(UNITS) |                                          | LOSS OF<br>BILIRUBIN WITH<br>VAN DEN BERGH<br>METHOD<br>(PER CENT) | REMARKS                                                          |
|-----------------------------|------------------------------------------|--------------------------------------------------------------------|------------------------------------------------------------------|
| VAN DEN BERGH<br>METHOD     | THANNHAUSER<br>AND<br>ANDERSEN<br>METHOD |                                                                    |                                                                  |
| 4.8                         | 5.2                                      | 8                                                                  | No skin jaundice                                                 |
| 12.0                        | 14.4                                     | 17                                                                 | Definite skin jaundice                                           |
| 19.0                        | 19.4                                     | 2                                                                  | Marked jaundice                                                  |
| 22.0                        | 20.4                                     | 0                                                                  |                                                                  |
| 15.0                        | 16.8                                     | 11                                                                 | Depth of jaundice stationary                                     |
| 8.0                         | 10.4                                     | 23                                                                 | Jaundice commencing to fade                                      |
| 5.5                         | 9.6                                      | 43                                                                 |                                                                  |
| 4.0                         | 6.4                                      | 37                                                                 |                                                                  |
| 2.0                         | 5.3                                      | 62                                                                 | Jaundice rapidly subsiding, but marked<br>jaundice still present |

TABLE III

SHOWING A SERIES OF DETERMINATIONS IN THE SAME INDIVIDUAL  
HOSPITAL No. 3251/33. DIAGNOSIS: OBSTRUCTIVE JAUNDICE—CARCINOMA OF HEAD OF  
PANCREAS

| PLASMA BILIRUBIN<br>(UNITS) |                                          | LOSS OF<br>BILIRUBIN WITH<br>VAN DEN BERGH<br>METHOD<br>(PER CENT) | REMARKS                                                |
|-----------------------------|------------------------------------------|--------------------------------------------------------------------|--------------------------------------------------------|
| VAN DEN BERGH<br>METHOD     | THANNHAUSER<br>AND<br>ANDERSEN<br>METHOD |                                                                    |                                                        |
| 12.0                        | 16.8                                     | 29                                                                 |                                                        |
| 13.0                        | 15.6                                     | 17                                                                 |                                                        |
| 14.0                        | 14.4                                     | 3                                                                  |                                                        |
| 12.0                        | 16.8                                     | 29                                                                 |                                                        |
| 14.0                        | 16.8                                     | 17                                                                 | Jaundice remaining stationary to this time             |
| 21.0                        | 26.4                                     | 20                                                                 |                                                        |
| 3.5                         | 7.2                                      | 51                                                                 | Jaundice subsiding following cholecystenter-<br>ostomy |
| 1.7                         | 3.6                                      | 53                                                                 |                                                        |
| 1.8                         | 3.8                                      | 53                                                                 | Very slight skin jaundice still present                |
| 0.8                         | 1.8                                      | 55                                                                 | No skin jaundice                                       |
| 0.6                         | 1.0                                      | 40                                                                 |                                                        |



TABLE IV  
SHOWING A SERIES OF DETERMINATIONS IN THE SAME INDIVIDUAL  
HOSP. NO. 3516/33. DIAGNOSIS: CATARRHAL JAUNDICE

| PLASMA BILIRUBIN<br>(UNITS) |                                          | LOSS OF<br>BILIRUBIN WITH<br>VAN DEN BERGH<br>METHOD<br>(PER CENT) | REMARKS                      |
|-----------------------------|------------------------------------------|--------------------------------------------------------------------|------------------------------|
| VAN DEN BERGH<br>METHOD     | THANNHAUSER<br>AND<br>ANDERSEN<br>METHOD |                                                                    |                              |
| 32.0                        | 31.2                                     | 0                                                                  |                              |
| 24.0                        | 24.0                                     | 0                                                                  | Depth of jaundice stationary |
| 11.0                        | 13.2                                     | 17                                                                 | Jaundice commencing to fade  |
| 4.7                         | 8.8                                      | 47                                                                 | Jaundice rapidly subsiding   |
| 4.0                         | 9.2                                      | 56                                                                 |                              |
| 1.3                         | 4.8                                      | 73                                                                 | Still slight jaundice        |

The observation that the van den Bergh technic is not even approximately quantitative in subsiding jaundice explains largely the absence of relationship noted at times between the clinical picture (color of the skin) and the bilirubin content of the blood in this type of case. With the Thannhauser and Andersen technic the discrepancy is not nearly so marked, although there is still evidence that, at times, the rate at which bilirubin enters the blood from the tissues is lower than the rate at which it can be removed. In all other types of cases investigated, the van den Bergh technic, although not strictly quantitative, is sufficiently so for clinical purposes. It may here be noted that it was found that in the detection of "latent" jaundice, both technics are equally sensitive.

#### SUMMARY

One hundred determinations were made of the bilirubin content of blood by the van den Bergh and the Thannhauser and Andersen methods in cases with "direct" bilirubin. Both methods were used in each case and determinations were made simultaneously.

In subsiding jaundice, the values obtained by the van den Bergh technic are not even approximately correct; loss of bilirubin may be as much as 60 per cent or more. In such cases, therefore, the Thannhauser and Andersen procedure is indispensable. In all other cases, however, including latent jaundice, the van den Bergh technic, although not strictly quantitative, is sufficiently so for clinical purposes.

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## A METHOD OF PREPARING DUODENAL POUCHES FOR USE IN EXPERIMENTAL WORK\*

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DURING the course of experimental investigations of the secretions found in the duodenum, it sometimes becomes desirable to prepare an isolated segment of the duodenum, one end of which opens on the abdominal wall. By means of such a pouch studies may conveniently be made of the duodenal contents, uncontaminated by juices from the stomach or jejunum. If the openings of the bile and pancreatic ducts are included in the segment of duodenum utilized for the pouch, as is usually the case, certain difficulties present themselves. As Elman and McCaughan<sup>1</sup> have shown, the duration of life after a fistula of the pancreatic ducts has been established is a matter of only a few days, and the animal will thus not live long enough to permit firm healing of the closed end of the pouch. During the first week there is apt to be an inflammatory reaction at the suture line which might interfere with normal experimental results, and in addition if fluid is introduced into the pouch under any degree of pressure there may be leakage at the suture line. We have had occasion to prepare and use a number of duodenal pouches,<sup>2</sup> and have found that the above factors are eliminated if the pouch is made by the following method:

Under intratracheal ether anesthesia, and using aseptic technic, the abdomen is opened through a midline incision extending from the ensiform cartilage downward about 4 inches. (We have used the dog in all of our work.) The first part of the jejunum is identified and marked by an Allis forcep. The pyloric end of the stomach and the first portion of the duodenum are brought up, packed off, and severed between heavy clamps at the pylorus. The end of the stomach and the adjacent end of the duodenum are then inverted and closed by two layers of 00 chromic catgut. The previously marked jejunum is then approximated to the anterior surface of the stomach and a gastroenterostomy is performed. The abdomen is closed in the usual manner. This constitutes the first stage of the operation, and the animal is now allowed a period of recuperation, usually about two weeks, before the second procedure. During this time

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the closed end of the duodenum, which will be the blind end of the pouch, has an opportunity to heal completely, and the animal returns to a normal physical condition.

The second stage of the operation requires only a few minutes and is performed as follows: The abdomen is again opened through a part of the length

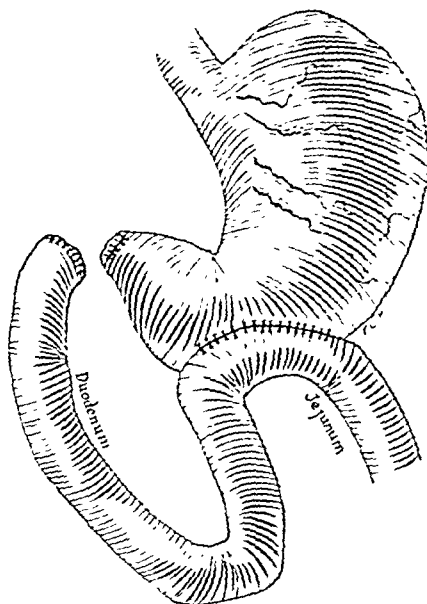


Fig. 1.—The first stage in the preparation of a duodenal pouch. The duodenum has been severed from the stomach at the pylorus, the open ends have been closed, and an anterior gastroenterostomy has been performed.

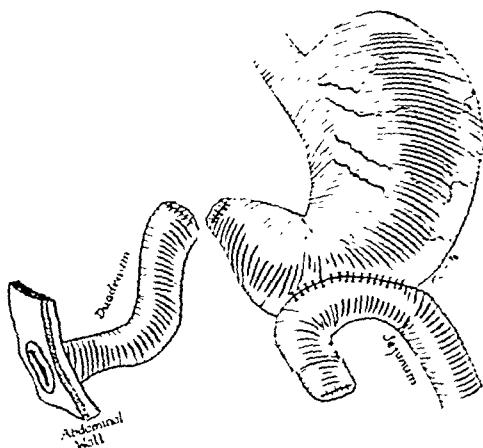


Fig. 2.—At the conclusion of the second stage of the operation: the duodenum has been cut off almost 6 inches from the pylorus and the open end has been brought through the abdominal wall. The distal duodenal stump has been closed.

of the original incision, and the duodenum is brought up into the wound. The field is packed off, and crushing clamps are placed on the duodenum about six inches from the closed end. The duodenum is severed between the clamps, and the stump nearest the jejunum is inverted and closed by two layers of

00 catgut or fine silk. A stab wound is made through the right rectus muscle near the costal margin, an Allis forcep is inserted into the abdomen through the stab wound, the crushing clamp is removed and that end of the duodenum is grasped and drawn out. The agglutination of tissues produced by the heavy clamp keeps the end of the duodenum closed while it is being drawn out, and there should be no soiling by duodenal juices. The open end of the duodenum is anchored to the peritoneum, fascia, and skin, care being taken not to interfere with the blood supply. The abdominal wound is closed again by the usual method. There is usually some shrinkage of the pouch, but we have found that if it is originally made 6 inches long, it will remain satisfactory. Most of our pouches held about 30 c.c. The pouch, when completed, retains its original nerve supply and the blood supply is, of course, very carefully preserved.



Fig. 3.—Photograph of the dog's abdomen showing the stoma of the pouch with the catheter and rubber stopper in place. Fluid is being withdrawn from the pouch by means of a syringe.

Owing to the extreme rapidity with which the physical condition of the animal deteriorates as soon as the bile and pancreatic juices begin to drain to the outside, it is advisable to start the proposed investigations within a short period of time. We are accustomed to begin the first series of experiments within eighteen hours after the completion of the operation. In order to keep the animal in good physical condition as long as possible, daily injections of 500 to 1,000 c.c. of 2 per cent sodium bicarbonate in normal saline solution should be given daily. We have found that fluid given subcutaneously in the dog is absorbed rapidly and can be given with ease and in large amounts. If the animal is in a debilitated condition, it is frequently difficult and time consuming to give fluid intravenously each day, and the subcutaneous route has given perfect satisfaction.

In a pouch prepared by the above method, with the duodenum brought out through the rectus muscle, there would in time be some valve action developed

at the stoma as the mucosa shrinks down. When the pouch is used early, however, and the mucosa still protrudes some distance from the wound, we have found it necessary in most cases to use a mechanical method of insuring that there will be no loss of fluid during an experiment. A rubber catheter, size 16 French, is passed through a rubber stopper of such size as to occlude the opening of the pouch. The catheter and stopper are adjusted so that when the stopper is in place in the stoma the catheter will project about 2 inches into the pouch. By means of a syringe, fluid may now be introduced into or withdrawn from the pouch without any leakage, especially if the stopper is held in place by manual pressure. In some cases it may be found that there is oozing of blood from the protruding mucosa in the earlier experiments, before any healing has taken place. If this might invalidate the experimental data, the bleeding may be easily stopped by touching the hemorrhagic edges with the cautery.

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## RETICULOCYTES\*

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RETICULOCYTES are young red cells, and therefore, a reticulocyte count gives the best evidence of the state of activity of the bone marrow. It is indicated in the study of any anemia and is especially important if pernicious anemia, aplastic anemia, or familial hemolytic icterus is being considered. An increase in the reticulocytes is the first indication of a favorable effect from therapy in anemias, whether that therapy be liver, iron, or any erythropoietic stimulant. Since the reticulocyte count is so important and is so frequently done, it is desirable to have a simple and accurate method which will give uniform results. It is evident from the large number of methods in use and from the wide variation (from as low as 0 to 0.1 per cent<sup>1</sup> to as high as 1.0 to 3.0 per cent<sup>2</sup>) in values given for normals, that a uniformly satisfactory method has not yet been devised.

While attempting to find the most desirable method of those now in use, a technic was worked out which seemed even more simple and showed more reticulocytes in a particular blood than any other method tested. It has the

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additional advantage of being adapted for use with oxalated venous blood in our uniform system<sup>3</sup> of hematologic methods. Evidence was further adduced to show that the reticulocyte stain is not a vital stain, as is usually stated, but will stain dead cells, if they are not fixed.

#### STUDY OF METHODS

Methods for reticulocyte staining may be classified in the following groups:

1. Methods in which the dye is dried on the cover slip or slide.
  - (a) Moist cover slip preparations.
  - (b) Dried smears.
2. Methods in which the blood is mixed with a solution of the dye.
  - (a) On the slide or cover slip.
  - (b) In test tube, pipette, watch glass, etc.

Obviously, the concentration, time of exposure to, and nature of the dye used may be varied with each one of these fundamental technics. Various dyes have been tried, but brilliant cresyl blue has been found so superior that the others need not be considered.

A series of experiments were performed to decide which of the above fundamental technics was most satisfactory. The methods were selected from the current literature and the textbooks in common use.

Blood was drawn from the vein of a patient with pernicious anemia and used for Methods A to F below and the remainder mixed with 2 mg. of potassium oxalate per cubic centimeter of blood and used for our experiments with oxalated blood. Five hundred red cells were counted in each experiment.

Method A: A small drop of fresh blood was placed on a cover slip, ringed with vaseline to prevent drying, and inverted onto a slide which had been smeared with a drop of saturated alcoholic solution of brilliant cresyl blue and allowed to dry. These were examined after thirty minutes. Some reticulocytes could be seen, but the smears were too thick and the red cells too unevenly distributed for accurate counting.

Method B: Smears of fresh blood were made on slides which had been smeared with a drop of saturated alcoholic solution of brilliant cresyl blue and allowed to dry. These smears gave a count of 7.6 per cent reticulocytes, which seemed to be well stained.

Method C: One drop of fresh blood was added to one drop of stain on a slide, mixed and smeared. The stain was made by mixing 2 c.c. of 1 per cent aqueous brilliant cresyl blue and 8 c.c. of 0.2 per cent potassium oxalate in 0.6 per cent sodium chloride solution, giving a final concentration of 0.2 per cent brilliant cresyl blue, 0.18 per cent potassium oxalate and 0.48 per cent sodium chloride. These smears gave a count of 8.2 per cent reticulocytes, which seemed to be well stained.

Method D: Fresh blood was drawn up to the 1 mark in a white cell pipette and filled with stain to the 11 mark and mixed thoroughly. After standing fifteen minutes, the pipette was shaken for two minutes and smears made, drying above a flame. The stain was made by mixing 3 parts of a 0.3 per cent solution of brilliant cresyl blue in 0.85 per cent sodium chloride and 1 part 0.3 per cent sodium citrate in 0.6 per cent sodium chloride, giving a final concentration of 0.075 per cent brilliant cresyl blue, 0.075 per cent sodium citrate, and 0.66 per cent sodium chloride. An occasional reticulocyte was seen, but the cells on these smears were crenated, making accurate counting impossible.

Method E: One drop of fresh blood was added to 20 drops of stain (prepared as in Method C) in a centrifuge tube, let stand for twenty minutes and centrifugated. Smears were

made of the sediment, drying over a flame. These smears gave a count of 9.6 per cent reticulocytes, which seemed to be well stained. The red cells were crenated in several of these smears.

Method F: Two drops of fresh blood were added to 25 drops of stain in a centrifuge tube, allowed to stand for twenty minutes, and then centrifugated. The stain was prepared by mixing 1 part of 1 per cent brilliant cresyl blue in 0.85 per cent sodium chloride and

TABLE I

| METHOD | RETICULOCYTES     |
|--------|-------------------|
| A      | Poor distribution |
| B      | 7.6%              |
| C      | 8.2%              |
| D      | Crenated          |
| E      | 9.6%              |
| F      | 17.6%             |
| G      | 23.6%             |

5 parts of 1.0 per cent potassium oxalate in 0.85 per cent sodium chloride, giving a final concentration of 0.17 per cent brilliant cresyl blue, 0.17 per cent potassium oxalate, and 0.85 per cent sodium chloride. Smears were made of the sediment, drying over a flame. These smears gave a count of 17.6 per cent reticulocytes, which seemed to be well stained. There was a tendency toward crenation of the cells.

From these experiments, it was concluded that the fundamental Method 2(b) is much the most satisfactory because it permits more accurate control of the concentration of dye with which the cells come in contact and of the time of exposure of the cells to the dye, and because the technic is simple and gives permanent preparations if counterstained with Wright's stain. The wide variation in counts on the same blood specimen by the different methods (Table I) proved that accurate control of these factors is necessary, and that most, if not all, of these methods fail to demonstrate all the reticulocytes actually present.

Further experiments were planned to find out whether oxalated blood could be used and to determine the optimum concentration of dye and time of exposure. Using Method F, which gave the highest count with the fresh blood (17.6 per cent) and the oxalated blood which had stood at room temperature for one hour, a count of 14.6 per cent reticulocytes was obtained, proving oxalated blood to be satisfactory. By using equal parts of oxalated blood and dye solution, satisfactory smears were obtained without centrifugation. Since the blood already contained an anticoagulant, the oxalate solution was omitted and mixing the blood with an equal volume of brilliant cresyl blue solution in 0.85 per cent sodium chloride was tried. Using a series of dye concentrations from 0.075 per cent to 14.0 per cent (saturated), it was found that a 1.0 per cent solution gave the maximum number (23.6 per cent) of reticulocytes with the minimum amount of debris on the slide. Concentrations below 1.0 or over 3.0 per cent gave low counts; the concentrations above 1.0 up to 3.0 per cent gave quite satisfactory smears when counterstained with Wright's stain. Using equal parts of the 1.0 per cent dye solution and oxalated blood, the time of exposure to the dye was varied from mixing on the slide (fifteen seconds)

to twenty-four hours, and it was found that results were satisfactory for all times over one minute, but that low counts were obtained if the time were thirty seconds or less. Red cell counts made on the original blood and on an accurate dilution with the dye solution showed that no hemolysis occurred.

From these experiments, it was concluded that the following is the most satisfactory technic for staining and counting reticulocytes: Mix, in a small test tube, equal parts (5 drops) of oxalated venous or capillary blood and 1 per cent of brilliant cresyl blue in 0.85 per cent sodium chloride solution. Let stand at least one minute, mix, and make thin smears, drying in the air as usual. These smears may be counted at any time within twenty-four hours, but if a permanent preparation is desired, they should be counterstained with Wright's stain by the usual technic. The brilliant cresyl blue solution keeps well but should be filtered if débris appears on the slide. Select an area on the slide which contains 50 to 75 red cells per oil immersion field. Count all the cells and all the reticulocytes in as many adjacent fields as is necessary to give a total of 500 red cells if the count is over 5 per cent, or 1,000 red cells if the count is less than 5 per cent reticulocytes. With this method, a count of 23.6 per cent was obtained in the blood which gave 17.6 per cent with the best method previously studied.

Using this technic (Method G) reticulocyte counts were made at intervals for twenty-four hours after drawing blood, and then at twenty-four-hour intervals up to ninety-six hours, and again at one hundred and forty-four hours. To our surprise, the counts showed no significant variations during the six days (Table II), although after ninety-six hours the blood was badly hemo-

TABLE II

| TIME AFTER DRAWING<br>BLOOD | RETICULOCYTES<br>PER CENT |
|-----------------------------|---------------------------|
| 1 hr., 30 min.              | 22.6                      |
| 3 hr.                       | 19.2                      |
| 5 hr.                       | 27.6                      |
| 23 hr.                      | 22.8                      |
| 24 hr.                      | 23.2                      |
| 48 hr.                      | 27.0                      |
| 72 hr. (some hemolysis)     | 23.8                      |
| 96 hr. (marked hemolysis)   | 26.8                      |
| 144 hr. (marked hemolysis)  | 28.0                      |
| Average                     | 23.6                      |

TABLE III

| TIME    | OXALATED BLOOD<br>RETICULOCYTES<br>PER CENT | OXALATE + FLUORIDE<br>RETICULOCYTES<br>PER CENT |
|---------|---------------------------------------------|-------------------------------------------------|
| 5 min.  | 11.40                                       |                                                 |
| 30 min. |                                             | 10.20                                           |
| 2 hr.   | 10.80                                       | 8.85                                            |
| 3 hr.   | 8.00                                        | 10.80                                           |
| 26 hr.  | 10.80                                       | 12.00                                           |
|         | 12.20                                       | 8.80                                            |
| Average | 10.64                                       | 10.13                                           |



TABLE IV

| RETICULOCYTES |            | RETICULOCYTES |            |
|---------------|------------|---------------|------------|
| PER CENT      | PER C. MM. | PER CENT      | PER C. MM. |
| Men           |            | Men           |            |
| 0.5           | 26,050     | 1.4           | 74,620     |
| 0.6           | 28,320     | 1.5           | 65,850     |
| 0.6           | 30,120     | 1.5           | 72,300     |
| 0.6           | 32,220     | 1.5           | 81,450     |
| 0.6           | 34,620     | 1.5           | 81,900     |
| 0.6           | 36,180     | 1.5           | 82,200     |
| 0.7           | 29,890     | 1.5           | 92,850     |
| 0.7           | 33,600     | 1.5           | 73,760     |
| 0.7           | 33,600     | 1.6           | 82,880     |
| 0.7           | 34,720     | 1.6           | 86,400     |
| 0.7           | 35,730     | 1.6           | 86,560     |
| 0.7           | 35,770     | 1.6           | 87,360     |
| 0.7           | 37,100     | 1.6           | 80,920     |
| 0.7           | 37,170     | 1.7           | 93,670     |
| 0.7           | 37,940     | 1.7           | 100,810    |
| 0.7           | 40,250     | 1.7           | 97,200     |
| 0.8           | 32,960     | 1.7           | 98,460     |
| 0.8           | 37,040     | 1.8           | 107,460    |
| 0.8           | 41,120     | 1.8           | 107,640    |
| 0.8           | 43,360     | 1.8           | 108,180    |
| 0.8           | 43,630     | 1.8           | 111,780    |
| 0.8           | 50,800     | 1.8           | 95,190     |
| 0.9           | 38,970     | 1.9           | 102,600    |
| 0.9           | 40,850     | 1.9           | 103,550    |
| 0.9           | 44,730     | 1.9           | 98,000     |
| 0.9           | 45,090     | 2.0           | 106,200    |
| 0.9           | 46,620     | 2.0           | 107,600    |
| 0.9           | 47,610     | 2.0           | 116,600    |
| 0.9           | 49,680     | 2.0           | 104,580    |
| 0.9           | 49,950     | 2.1           | 106,890    |
| 0.9           | 52,200     | 2.1           | 104,940    |
| 1.0           | 50,200     | 2.2           | 111,100    |
| 1.0           | 51,500     | 2.2           | 111,760    |
| 1.0           | 51,900     | 2.2           | 114,770    |
| 1.0           | 52,100     | 2.2           | 115,230    |
| 1.1           | 52,900     | 2.3           | 123,970    |
| 1.1           | 52,140     | 2.3           | 120,000    |
| 1.1           | 54,000     | 2.3           | 126,720    |
| 1.1           | 55,440     | 2.4           | 140,000    |
| 1.1           | 56,100     | 2.4           | 136,240    |
| 1.1           | 58,300     | 2.5           | 156,520    |
| 1.1           | 58,850     | 2.6           | 137,200    |
| 1.1           | 59,070     | 2.6           | 144,130    |
| 1.1           | 61,490     | 2.8           | 175,450    |
| 1.2           | 53,160     | 2.9           | 195,870    |
| 1.2           | 53,880     | 2.9           | 133,200    |
| 1.2           | 55,800     | 3.0           | 184,500    |
| 1.2           | 58,680     | 3.0           | 148,800    |
| 1.2           | 61,560     | 3.1           | 171,200    |
| 1.2           | 62,640     | 3.2           | 165,660    |
| 1.2           | 67,400     | 3.3           | 172,720    |
| 1.3           | 70,200     | 3.4           | 201,620    |
| 1.3           | 70,850     | 3.4           | 211,660    |
| 1.3           | 77,090     | 3.8           |            |
| 1.4           | 78,910     |               |            |
| 1.4           | 70,560     |               |            |
| 1.4           | 70,700     |               |            |
| Average       |            | 1.57          | 83,160     |
| Minimum       |            | 0.50          | 26,050     |
| Maximum       |            | 3.80          | 211,660    |

TABLE IV—CONT'D

| RETICULOCYTES |            | RETICULOCYTES |            |
|---------------|------------|---------------|------------|
| PER CENT      | PER C. MM. | PER CENT      | PER C. MM. |
| Women         |            | Women         |            |
| 0.5           | 21,400     | 1.5           | 75,300     |
| 0.5           | 22,300     | 1.6           | 65,280     |
| 0.8           | 37,120     | 1.6           | 72,800     |
| 0.9           | 36,450     | 1.6           | 73,600     |
| 0.9           | 41,040     | 1.6           | 76,000     |
| 0.9           | 41,580     | 1.6           | 82,240     |
| 0.9           | 42,930     | 1.6           | 71,100     |
| 0.9           | 44,640     | 1.8           | 79,200     |
| 0.9           | 45,090     | 1.8           | 84,620     |
| 1.0           | 46,300     | 1.8           | 88,200     |
| 1.0           | 46,500     | 1.9           | 92,950     |
| 1.0           | 47,000     | 2.0           | 91,400     |
| 1.0           | 49,600     | 2.1           | 106,470    |
| 1.1           | 44,000     | 2.2           | 96,360     |
| 1.1           | 49,390     | 2.2           | 100,320    |
| 1.1           | 49,610     | 2.2           | 107,800    |
| 1.1           | 50,710     | 2.3           | 103,960    |
| 1.2           | 53,160     | 2.5           | 121,500    |
| 1.2           | 56,880     | 2.6           | 120,120    |
| 1.2           | 60,120     | 2.6           | 131,300    |
| 1.3           | 51,740     | 3.3           | 150,150    |
| 1.4           | 51,180     | 3.5           | 156,800    |
| 1.4           | 69,860     | 3.6           | 153,360    |
| 1.4           | 72,800     |               |            |
| 1.5           | 63,200     | Average 1.57  | 72,800     |
| 1.5           | 66,900     | Minimum 0.50  | 21,400     |
| 1.5           | 67,950     | Maximum 3.60  | 156,800    |

lyzed. This showed that oxalated venous blood could be safely used for at least twenty-four hours, and suggested to us that this was not a vital stain, if the term "vital stain" is used in its strictest sense of a stain which acts only on living cells and in staining causes cell death. To aid in deciding this point, equal parts of oxalated blood and 1 per cent sodium cyanide were mixed and reticulocyte counts were done at intervals up to three hours. No significant variations in the reticulocyte counts from those on the untreated blood were found. Another sample of oxalated blood was mixed and divided into two parts. To one part sodium fluoride was added in the proportion of 10 mg. per c.c. Reticulocyte counts were made at intervals for twenty-four hours on both samples with the results shown in Table III. When the red cells were fixed by methyl or ethyl alcohol, formalin, or flame, the reticulocytes did not stain. The results of these experiments support the opinion<sup>4</sup> that the reticulum can be stained in unfixed, but not necessarily living, red cells.

#### NORMAL VALUES

To determine normal values for the improved technic, reticulocyte and red cell counts were done on 110 healthy young men and 50 healthy young women, with the results shown in Table IV. Note that the average figure is 1.57 per cent and the range from 0.5 to 3.8 per cent for both men and women. The total number of reticulocytes calculated from the red cell count averages 83,160 per c.mm. for men and 72,800 per c.mm. for women and shows a range of from

26,050 to 211,660 and from 21,400 to 156,800, respectively. Studies of normal hematology of over 200 children of school age show practically the same percentage values for reticulocytes. For clinical purposes, the average normal may be regarded as 1.5 per cent with figures below 0.5 or above 4.0 per cent, indicating decreased or increased red cell formation, respectively. Observe that these figures are significantly higher than those generally given and that reticulocytes were found in all normal bloods.

#### SUMMARY

A study of the methods for reticulocyte staining in common use showed a great number of different technics giving widely variant results.

A simple technic was worked out which gives uniformly good results and significantly higher counts than other methods tested.

Normal values for this method are 1.5 per cent with a range of 0.5 to 4.0 per cent. Since the percentage values for both sexes at all ages are the same, while the absolute counts differ markedly, it would seem better to report reticulocyte counts in percentage rather than in absolute numbers.

Evidence is presented which suggests that the term "vital" should not be used when referring to the reticulocyte stain.

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#### SMALL ANIMAL METABOLISM CAGE\*

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THE cage design presented here offers several advantages over the usual small animal cage. Among these advantages are (1) low cost per cage, the size given when completed averaging about forty cents each; (2) ease of construction, as no solder is necessary; (3) ease of cleaning; (4) immediate accessibility to the animals, which are visible at all times; (5) feed boxes and water founts which can be changed without opening or moving the cage; (6) the construction which

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permits of separation of urine and feces for examination; (7) the convenience of weighing animals without removing them from the cage.

The construction requires no special tools other than a pair of heavy shears, or tin snips, and pliers. The cage proper is a cylinder of  $\frac{5}{8}$ -inch mesh galvanized screen with a fitted bottom of the same material. Covers are made from  $8\frac{1}{2}$ -in. tin pot covers; the bottoms are tin pie pans of the same dimension. These items can readily be obtained from the local stores.

The cage size as used in our laboratories for rats is 10 in. high and 8 in. in diameter with the floor placed about  $2\frac{1}{2}$  in. from the cylinder bottoms. This height prevents coprophagy in vitamin work.

The mesh is cut so as to leave protruding mesh ends which are bent to form an L. These are clinched over in forming the cylinder as shown in Fig. 2, I. The floor of the cage may be wired to the walls as at *x* (Fig. 1), or stiff cross-

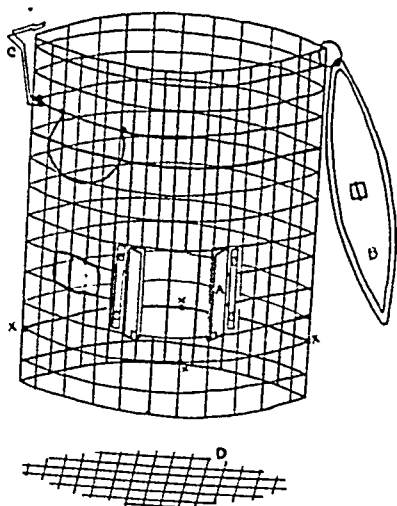


Fig. 1.

wires such as made from baling wire may be placed across the inside of the cylinder for the bottom to rest on. This latter procedure may allow of easier cleaning although some rigidity is lost by it. If the floor is made removable as suggested, it should be bound with tin strips. Such strips can be wired to the floor plate easily by punching holes in the binding strip. The cover hinge as well as the snap and water-fount support are made from baling wire. The cover hinge is an overlapped ring of the wire placed as shown.

The food tunnel is shaped from material obtained from discarded tin cans. These pieces were made into boxes about 10 cm. long by 5 cm. square with one end closed and the edges of the open end flared to fit the slots attached to the cage at *A* (Fig. 1). Small aluminum rivets were used to hold the box rigid and to attach the top of an ointment jar. The ointment jars or food jars are of porcelain or glass 6.5 cm. in diameter and 6 cm. high. By cutting an opening through the bottom of the feed tunnel and the attached ointment jar top, the animal is allowed ready access to the food. Spillage and wastage is thus cut

down, and the food jar may easily be removed for weighing, cleaning, and filling. If desired, the food tunnel opening can be fitted with tin squares which have holes of various sizes according to the rat size. In other experiments not requiring the accurate checking of food consumption, the slot *A* holds a plain piece of sheet tin and the food jars may be placed inside the cage. For daily checking of food weights, a glass of food or seed jar such as is used on canary cages may be used; this makes a more compact assembly, but these jars do not hold sufficient food for several days' feeding.

The water founts are the usual inverted bottles with L-shaped glass tubes having an opening blown on the upper surface of the lower limb as sketched at *H* (Fig. 2).

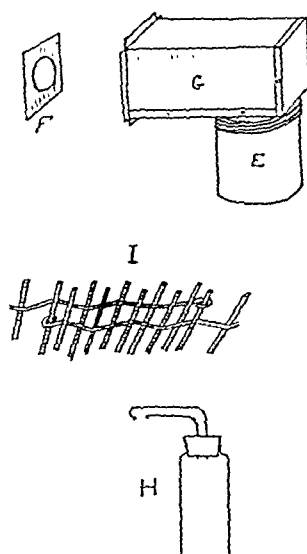


Fig. 2.

When using the cages in which separate urine and feces samples were desired, six-inch holes were bored in the shelf supporting the cages. Seven-and-a-quarter-inch glass funnels were placed in these holes and the cages placed over the funnels. Screens made from No. 60 brass strainer net were placed in the funnels to collect feces while the urine drained into small tubes or flasks placed at the funnel tips on a lower shelf. The funnel tip may be narrowed to deliver drops and a continuous record of urine secretion obtained by the use of methods suggested by Owen.<sup>1</sup> While we have routinely filtered urines before extraction in studies of drug excretion (Owen and Olsen<sup>2</sup>), this is usually not essential unless a dry, finely divided food is used.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**TUBERCULIN, Ointment Patch Test, Wolff, E.** *Am. J. Dis. Child.* 47: 764, 1934.

The author describes tests with an ointment composed of highly concentrated tuberculin and killed tubercle bacilli. The area of application is the paravertebral region between the eighth and eleventh thoracic vertebrae, or the medial brachial surface.

The skin is cleansed with green soap and water, dried gently, bathed with benzene, and then gently dried again. The application of benzene only, as heretofore practiced, is not sufficient to remove the secretions from very oily skins. A pea-sized drop of the tuberculin ointment is applied on the right side, and a similar sized drop of the control ointment is applied on the left side. Each of these drops is covered with a 2-inch (5 cm.) square of adhesive tape.

The test is read in forty-eight hours. The adhesive tape is removed after soaking with benzene, and the area is gently cleansed with benzene. The reaction may be clearly observed ten minutes after the removal of the tape.

Positive reactions exhibit papules, erythema, induration, and pigmentation. A weak positive test shows a few (not more than twenty) discrete papules from 1 to 3 mm. in diameter and pale rose in color. A medium reaction shows many vividly red papules surrounded by the normal skin. A strong reaction shows, in addition, changes of the total test area consisting in yellow brown pigmentation, indurative formation of plateaus, and marked reddening. A few or all of the papules may even show distinct formation of vesicles.

In weak reactions it is advisable to palpate the area in order to detect the follicles and to compare it with the area of the control.

The previously mentioned yellow pigmentation of the entire area is easily brought out by stretching the skin, whereas stretching the control area only blanches it.

The peak is reached after seventy-two hours, regardless of whether the test area was covered with adhesive tape for forty-eight or for seventy-two hours. This shows definitely that the reactive substances are absorbed by the skin within forty-eight hours and that the reaction of the skin to the absorbed material continues after the removal of the tape.

The color of the reaction begins to fade within ninety-six hours and is still faintly discernible up to one week. Medium reactions show a definite scaling up to approximately ten days; and in the marked reaction, even after two weeks, there may be roughening of the skin, brownish discolorations, infiltration, and scaling of the area.

In contradistinction to the intracutaneous test, there has never, thus far, been a generalized systemic reaction such as a rise in temperature, malaise, or focal signs.

Subjective symptoms accompanying the local reactions are mild. In nearly every positive case there is some itching over the test area, with only slight discomfort. In rare cases of very marked sensitiveness, slight pain over the test area may occur.

The ointment is stable and easier to apply, and it causes less psychic disturbance and fewer untoward general and focal reactions in the patient than does the old tuberculin used in the intracutaneous test.

The agreement between the ointment patch test and the intracutaneous test with tuberculin in all dilutions as performed on 190 children was 95.8 per cent.

The ointment patch test is generally comparable in results with an intracutaneous injection of 0.1 c.c. of old tuberculin, 1:100.

The ointment patch test produced markedly less nonspecific cutaneous irritation than the intracutaneous test.

**TUMOR GROWTH, "Heavy Water"** and, Woglom, W. H., and Weber, L. A. J. A. M. A. 102: 1289, 1934.

Deuterium, in the amounts that it was possible to administer as "heavy water," had no demonstrable effect on the growth of mouse sarcoma or mouse carcinoma.

**TETANUS: Development of Tetanus Antitoxin Following Administration of Tetanus Toxoid**, Sneath, P. A. T. J. A. M. A. 102: 1288, 1934.

Of 29 persons given three doses of tetanus toxoid, significant amounts of antitoxin developed in 28, a titer of at least 0.1 unit per cubic centimeter being reached in the majority, or 20.

From five to seven months after the last dose there was, in general, a reduction in the antitoxin level, but 27 still showed demonstrable antitoxin; and 25 showed 0.01 unit or more.

This suggests that active immunization with tetanus toxoid might be adopted advantageously by certain groups in whom the hazard of tetanus is greater than in the general population.

**MENINGITIS, Pneumococcic, Report of Case With Recovery Following Cisternal Drainage**, Bedell, C. C. J. A. M. A. 102: 820, 1934.

In a case of pneumococcic meningitis, Group IV, a drainage tube was inserted into the cisterna magna as soon as signs of meningeal invasion were observed. The tube remained in place for eight days; 200 c.c. of cerebrospinal fluid escaped daily by this route. Irrigations with physiologic solution of sodium chloride were carried out from the cisterna magna to the lumbar region. Periodic shrinkage of the brain volume was attempted by intravenous injections of hypertonic dextrose. The spinal fluid became secondarily infected with *Bacillus proteus* at the end of six days, producing a marked leucocytic response. There was progressive improvement, however, and the patient recovered completely and left the hospital at the end of seven weeks.

**GONOCOCCI, Preparation of Correct Picture and Simple Culture Method**, Szilvasi, J. Dermat. Wehnschr. Leipzig, 97: 1811, 1933.

To obtain the material for microscopic examination Szilvasi lets the patient urinate and then inserts a platinum loop into the anterior portion of the urethra and obtains a secretion that is readily spread out. After staining according to Gram's method, a striking picture is seen. Between monocytes and isolated threads of mucus, the gonococci are found in peculiar arrangements, in the form of coils, of clusters, or of wreaths or caps. The fact that an intensely stained zone surrounds the individual gonococcus enhances the peculiarity of the picture.

This method of obtaining the specimen of the urethral secretion is advantageous also for the culture of the gonococcus, a procedure which used to be rather difficult when the thick pus was used. The secretion is applied to the culture medium not in streaks, but in dots, and the gonococci are found on the margin of the dots. The facts that in the pus the majority of gonococci are either destroyed or impaired and that the phagocytic leucocytes contain other microorganisms are probably the reasons why the demonstration and culture are less successful when thick pus is used than when the secretion is used.

**ANEMIA, Aplastic, Greenwald, H. M.** Am. J. Dis. Child. 47: 360, 1934.

It is now generally conceded that aplastic anemia is a disease *sui generis* and is not a biologic variation of some form of primary or secondary anemia.

The most characteristic pathologic finding is a sulphur yellow appearance of the bone marrow instead of the reddish appearance one would expect to find in severe anemia. This is

due to a replacement of the normal marrow by ordinary fat tissue. In the case reported, the bone marrow was of a cranberry red appearance. Histologic examination showed, however, that the reddish appearance of the bone marrow was due not to active regenerative processes, but to hemorrhage. Histologic examination revealed a total absence of nucleated red blood cells and myeloid elements.

The blood picture is, of course, the main point of clinical diagnosis. There is a marked decrease in the hemoglobin content, with a diminution in the red blood cells to 1,000,000 or less; the hemoglobin is reduced to 10 or 15 per cent. The color index is approximately 1. Nucleated red blood cells are usually absent although occasionally observers have found isolated normoblasts. Reticuloocytes and polychromatophilic erythrocytes are absent. There is leucopenia with a marked reduction of the neutrophilic leucocytes and an almost complete absence of eosinophils. There is a relative increase in the lymphocytes. The differential count often shows the presence of only from 8 to 10 per cent polymorphonuclear cells while the lymphocytes are frequently found to be 90 per cent or over. Thrombocytopenia is constant and is a feature of the disease. The coagulation time is normal, but the retraction of the clot is interfered with, and the bleeding time is prolonged. In addition to the absence of normoblasts and megaloblasts, myelocytes, myeloblasts, or other forms of embryonic cells are usually absent. The results of the fragility test are usually normal; Lucas, however, stated that the fragility of the red cells may be altered, owing to the inability of the bone marrow to produce new red cells.

The blood serum is water clear. There is no increase in bile pigments. Urobilinuria is absent, and there is no increase in the urobilin content of the stool.

The course of the disease is rapid, and it is usually fatal before four months have elapsed. In fact, some observers refuse to regard the case of a patient who lives longer than six months as a true case of aplastic anemia.

Up to the present no therapeutic measure has proved satisfactory in the treatment of this disease. Repeated transfusion is generally recommended, but it is, at best, only a palliative measure.

**BLOOD SEDIMENTATION:** The Interrelation of Cutler, Linzenmeier and Westergren Tests, Greisheimer, E. M., Treloar, A. E., and Ryan, M. *Am. J. M. Sc.* 187: 213, 1934.

Blood sedimentation in 99 men and 102 women, selected without regard to age or health from university students and ambulatory patients, has been studied to establish the interrelationships between the Linzenmeier, Cutler, and Westergren methods. The average sedimentation in one hour for "normal" subjects appears to be reasonably concordant for the three methods despite the wide differences in tube width, anticoagulant concentration and length of fluid column, although the differences between the means for the three methods are significant statistically. The average sedimentation at one hour for the women is approximately double that for the men.

The concordance between the results by each pair of methods for the individual patients has been subjected to analysis by preparing the correlation tables. The regression lines, for predicting the most likely value to be expected by any one test when that by another is known, proved to be of three distinct types. There is a rectilinear relationship between the Cutler index and the Linzenmeier index. The regressions between Westergren index and Cutler or Linzenmeier index appear to be somewhat like saturation curves. However, they had to be fitted by a systematic scheme of free-hand graduations for lack of a suitable type of mathematical equation. The relationships between the various indices and Linzenmeier time values may be suitably portrayed by section of hyperbolas. Tables are provided for these average predictions for each independent variable of clinical interest.

The dispersals of individual cases about the lines of average relationship, although not studied in detail, are clearly greater than those ascribable to errors inherent in the technics.



The conclusion is clear that sedimentation measures for human blood are, in part, specific for the technic employed.

Standardization of a generally acceptable method for blood sedimentation would prove most advantageous for clinical work.

**TUBERCULIN TEST:** Value of the Negative Intracutaneous (Mantoux) Test in Adults, Lichtenstein, M. R. *Am. Rev. Tuberc.* 39: 190, 1934.

Complete insensitiveness to tuberculo-protein makes it certain that the patient is non-tuberculous, with the few exceptions given.

Complete insensitiveness is found in a sufficient number of adults to make the test worth while.

*Patients who react only to the strong concentrations of tuberculo-protein, with the same exceptions, almost certainly have no active tuberculosis.*

The test quickly excludes as nontuberculous a number of suspects, including some of the most difficult cases for diagnosis.

The intracutaneous test, carried into the stronger concentrations, should be a part of the diagnostic routine of every physician considering tuberculosis.

**PNEUMONIA, in Newborn and Stillborn Infants, Warwick, M.** *Am. J. M. Sc.* 187: 253, 1934.

Pneumonia in newborn infants is a definite entity and should be recognized by obstetricians, pediatricians, and pathologists. It usually begins in utero or during birth. The majority of the victims have physical handicaps; consequently, the pneumonia may be the result of lowered resistance. The exciting cause is as yet uncertain but seems to rest between bacteria contaminating the amniotic fluid or an irritating amniotic fluid, particularly when it contains large amounts of bile and cornified epithelial cells. Present evidence seems to favor the latter.

The facts favoring bacteria as the cause of pneumonia in the newborn infant are given:

1. Bacteria are usually the cause of exudation of polymorphonuclear leucocytes and of pneumonia.
2. Bacteria can be demonstrated in some of the pneumonic lungs.
3. In some, possible sources of infection are found, such as premature rupture of the membranes or light respiratory infections in the mother.
4. Some of the cases of pneumonia occur in groups during the months in which respiratory infections are the most prevalent.
5. Amniotic fluid is very frequently found in lungs where there is no evidence of pneumonia.

On the other hand, equally good reasons may be found for the possibility of uninfected amniotic fluid being the etiologic factor and these are also given below:

1. Chemicals may cause an exudative inflammation and also pneumonia.
2. A large percentage of cases show no demonstrable bacteria.
3. Masses of aspirated amniotic fluid are often found surrounded by numerous polymorphonuclear leucocytes in the midst of an atelectatic lung.
4. The pneumonia is often associated with atelectasis.
5. Amniotic fluid may vary markedly in constituents and in irritability.
6. Infants with other physical handicaps are often affected.
7. In some cases no convincing possibility of entrance of bacteria is seen.

**TULAREMIA, Treated by a New Specific Antiserum, Foshay, L.** *Am. J. M. Sc.* 187: 235, 1934.

An initial trial has shown that the goat can be made to yield a potent antitularemia serum which is effective in treating tularemia in man. The intravenous administration of this serum produced a marked and prompt amelioration of symptoms in 14 out of 15

tularemia patients and caused a shortening of the duration of adenopathy, the period of disability, and the total duration of disease. One patient, received in dying condition with extensive involvement of the lungs, liver, and spleen, was not improved. The antiserum has a specific desensitizing action. There is evidence to show that its beneficial effects in man are intimately associated with, and perhaps dependent upon, this property.

**ARTHRITIS, Rheumatoid, Blair, J. E., and Hallman, F. A.** Arch. Int. Med. 53: 87, 1934.

In a series of 57 cultures of synovial fluids and tissues from chronic multiple arthritis (55 from rheumatoid arthritis and 2 from Still's disease), a total of 41 (74.5 per cent) remained sterile and 14 (25.4 per cent) yielded positive cultures. The microorganisms obtained in the positive cultures included streptococci, diphtheroid bacilli, gram-positive cocci incapable of growth on subculture, and an occasional gram-negative bacillus associated with the aforementioned bacteria.

In a series of 175 cultures of synovial fluids and tissues from a variety of chronic infectious and noninfectious conditions of the joint other than rheumatoid arthritis, 142 (81.1 per cent) were sterile and 33 (18.8 per cent) were positive. The positive cultures included *Staphylococcus aureus*, indifferent streptococci, gram-positive cocci incapable of growth on subculture, and diphtheroid bacilli.

No direct etiologic significance is attached to any of the organisms obtained in this series.

It is felt that at the present time no specific bacteriologic agent may be considered to have been demonstrated as the etiologic cause of rheumatoid arthritis, particularly in view of the multiplicity of results and the lack of general confirmation of any one report.

**BLOOD, in Normal Pregnancy, Dieckmann, W. J., and Wegner, C. R.** Arch. Int. Med. 53: 71, 1934.

Previously reported values for blood and plasma volumes in pregnancy are at variance with each other, and the results are inconclusive because of the different methods used and the method of calculation. The volumes are reported either in cubic centimeters per kilogram or in percentage of body weight, either of which is unreliable because of the constantly changing weight in pregnancy.

Determinations of the blood and plasma volumes were made on various groups of women for the different periods of pregnancy. The cubic centimeters per kilogram and the means for the different periods were calculated, and although there is a slight increase at term, statistical analysis indicates that the changes are of no significance.

Similar studies in which the same women were followed throughout pregnancy and the puerperium indicate that the following changes occur:

1. The blood and plasma volumes begin to increase in the first trimester, and by the thirteenth week the gain amounts to 16 per cent and 18 per cent, respectively.

2. At term the average increase in the blood volume is 23 per cent and the plasma 25 per cent. This change is designated an "oligoeythemie hypervolemia." Although this increase seems large, losses of blood of 700 c.c. or more are at once manifested in measurable reductions in volume. The pregnant woman survives losses of blood which would be fatal if she were not pregnant, partly because of the increase in blood volume, but more particularly because of the tremendous amount of fluid in her tissues.

3. At eight weeks postpartum there is an average decrease of 16 per cent for both blood and plasma volumes. This does not quite equal the increase, but, since most of the patients weigh more after pregnancy, the discrepancy is explained.

4. The increase in blood and plasma volumes is not merely to fill vessels but is probably a part of the mechanism required to permit proper fetal respiration.

**PNEUMONIA**, Epidemiology of Lobar, Smillie, W. G., and Leeder, F. S. *Am. J. Pub. Health* 24: 129, 1934.

Nasopharyngeal cultures from 264 contacts of 64 cases of lobar pneumonia due to Type I and Type II pneumococcus have been studied. The results indicate that 20 per cent of the immediate family contacts of these patients harbored the homologous strain in their nasopharynges. The hospital contacts of the same patients, however, were seldom infected (about 2 per cent). These results suggest that it is quite justifiable to treat cases of lobar pneumonia due to Type I and Type II pneumococci in the open wards of general hospitals.

One experiment indicates that a healthy carrier of Type I pneumococcus does not transmit this organism to immediate contacts, even though the individuals are living under overcrowded conditions.

The evidence suggests that there is some additional factor other than simple contact which determines the transfer of Type I or II pneumococci from a patient with lobar pneumonia to contacts. Nineteen of the 25 family contacts of cases of lobar pneumonia due to Type I or Type II pneumococci that became homologous carriers were suffering from acute colds at the time the cultures were taken. Positive cultures were found as frequently on the first day of exposure as after a week. This evidence suggests that family epidemics of colds may be a factor which determines the transfer and establishment of Type I and Type II pneumococci from the infected to the uninfected.

Carriers of Type I and Type II pneumococci, when once established, may continue as carriers of these strains for a considerable period of time without giving rise to lobar pneumonia in themselves or their contacts and without producing a second group of carriers.

**MILK**, Examination of for Members of the *Escherichia-Aerobacter* Group, McCrady, M. H., and Archambault, J. *Am. J. Pub. Health* 24: 122, 1934.

Comparative results obtained from examination of milk samples for *Escherichia-Aerobacter* by various methods suggest the following tentative conclusions:

1. So-called "typical" colonies appearing in solid media pour-plates inoculated with milk may not be accepted as colonies of *Escherichia-Aerobacter* unless a sufficiently thorough examination of a large number of colonies from representative samples has proved that they may be so accepted. In the hands of the authors, plates poured with neither Endo, bile salt, nor eosin methylene blue agar proved reliable for direct enumeration of colon organisms in milk samples since the proportion of resulting typical colonies which were confirmed varied from zero in some samples to 100 per cent in others. It is quite probable that similar difficulties would be encountered in the examination of dairy products other than milk by these plate methods.

2. The production of 10 per cent or more gas in brilliant green bile 2 per cent special fermentation tubes inoculated with quantities of raw or pasteurized milk varying from 10 c.c. to 0.001 c.c. and incubated forty-eight hours at 37° C. provides reasonable assurance of the presence of *Escherichia-Aerobacter* organisms. This medium appears to be as reliable as gentian violet bile for the enumeration of these organisms and is more conveniently employed in those laboratories where it is also used for examination of water samples. The percentage of such brilliant green bile presumptives which were completely confirmed varied as follows:

|                                        |              |
|----------------------------------------|--------------|
| Raw milk, 1 c.c. to 0.001 c.c.         | 99 per cent  |
| Pasteurized milk, 10 c.c.              | 100 per cent |
| Pasteurized milk, 1 c.c. to 0.001 c.c. | 86 per cent  |

It is pointed out that since the organisms in 10 c.c. of properly pasteurized milk rarely produce gas in this medium, the production of any amount of gas from the majority of such samples of 10 c.c. or less of a pasteurized milk is usually indicative of some fault in the processing of the milk, either improper pasteurization or recontamination of the pasteurized product.

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## EDITORIAL

### Developments in the Study of Agglutinins

THE bacteriologist of the future will, without doubt, look back with envy upon the days when dogma was supreme, when bacterial species were immutable, and any variation from the accepted type was dismissed with complacence as an "involution form."

Indeed, it is quite possible that this attitude may even be encountered among the bacteriologists of today, confronted as they are with a confused array of "rough" and "smooth" colonies with H's and O's thrown in for good measure!

At first regarded as of purely academic interest, later studies have shown quite definitely that these bacterial variations have a very definite and perhaps wide application to the practical problems of bacteriology, immunity, and epidemiology and cannot be disregarded in the utilization of agglutination reactions in the study of disease.

Among the oldest and perhaps also the most commonly applied agglutination test is that devised by Widal for the study of typhoid fever; certainly it is perhaps the one most familiar to the practitioner at large and because of the fact that large numbers of the general population have now a history of antityphoid vaccination as an aftermath of the World War, general concepts as to the clinical interpretation of positive Widal reactions have, perforce, been the subject of renewed study and modification which it may be of some interest to summarize and review.

The occurrence of agglutinins for the typhoid group, as well as for other organisms, in nonvaccinated, supposedly normal individuals has been studied by numerous investigators interested, not only in their occurrence but in the explanation of their presence.

Perhaps the majority of observations in this field support the view that the occurrence of such agglutinins is the result of previous exposure to the specific infectious agent, either through vaccination or as a result of natural clinical or subclinical infection.

Havens and Mayfield<sup>1</sup> in a study of 1,136 such individuals encountered 263 positive reactions or 23 per cent, 14 per cent in a dilution of 1:40; 6 per cent in a dilution of 1:80, and 3 per cent in a dilution of 1:160, results comparable to those of other and previous investigators.

These so-called "normal" agglutinins behaved in the same manner and were in all respects indistinguishable from specific antibodies. As in only 60 of the 263 positive reactors was it possible to obtain any information concerning any previous attack of clinical typhoid fever or of prior vaccination, 48 having no such history, it must be assumed that, if these 60 may be taken as a fair sample of the group, only 20 per cent of all positive Widal reactions represent the aftermath of vaccination or clinical typhoid fever.

Other observers, however, have reported the apparent nonspecific production of typhoid agglutinins. For example, Kilduffe and Hersohn<sup>2</sup> demonstrated the presence of typhoid agglutinins in 29 per cent of 200 serums from tuberculous patients, only 33 per cent of those reacting having any history of previous vaccination or clinical typhoid fever.

In the interpretation of such reactions various possibilities must be taken into account: They may be either nonspecific or heterologous; they may represent the result of an unrecognized or subclinical typhoid infection in the past and, as such, an expression of the reawakening of a previous specific antigenic stimulus, or they may exemplify, as indicated by the observation of Gilbert and Coleman,<sup>3</sup> the fact that exposure to certain bacterial types will again initiate production of apparently unrelated antibodies such as have developed previously in response to an antigenic stimulus of different nature.

While there may be room for discussion as to the nature and mechanism of such reactions, there can be no question that they may definitely complicate the interpretation of diagnostic agglutination tests.

In view of the findings above referred to it is, or should be, obvious that the trend of a series of agglutination reactions furnishes evidence much more clear-cut and definite than that obtainable from a single test, just as the trend

of a series of leucocyte counts is of far greater value than the results of a single determination. Nevertheless, in by far the greater majority of instances, clinical investigations do not include such serial studies and clinical inferences, and deductions must stand or fall upon the results of a single agglutination test.

Under these circumstances it is fortunate that studies in bacterial mutation and variation have demonstrated a relation between this and the response of the organisms to agglutinins and that evidence is accumulating which suggests that even a single agglutination test may furnish reliable information when interpreted in the light of these newer investigations.

That variations may occur in colonies of a pure culture of a single bacterial species was long ago recorded, but it is only within comparatively recent times that it has been appreciated that these variations are indicative of variations in antigenic structure and of variation in the production and the response to agglutinins.

The starting point of these investigations may be said to be the work of Weil and Felix<sup>1</sup> who first demonstrated the existence of dual antigens in many bacteria and showed their relation to variations in the character of their colonies, for while the observation had been recorded before by the English workers Smith and Reagh and Beyer and Reagh, it passed almost unnoticed until the work of Weil and Felix in 1917 and their later publication in 1924 demonstrated its practical importance in the interpretation of agglutination reactions.

During the study of the anomalous Weil-Felix reaction (the agglutination of a particular strain, *Proteus* X19, of proteus organisms by the blood of patients suffering from typhus fever), these workers recorded first, that there occurred two types of colonies in the proteus cultures; second, that these two types presented differing agglutinogenic properties, and, finally, that the clinical interpretation of agglutination reactions was affected by and related to these differences.

The normal, flagellated proteus bacillus produces a thin, spreading growth spoken of as the "exhalation" (Hauch) form, while the nonflagellated variant presented isolated colonies without exhalation (Ohne Hauch), terms soon shortened to O and H.

Later developments demonstrated that the agglutinogenic properties of these O and H forms were different, the antigens—"H antigens" produced by the H forms being flagellar in origin and thermolabile while those produced by the O forms were thermostable and of somatic derivation.

The subsequent studies of numerous investigators showed that dual antigens were present in many bacteria among them the organisms of the typhoid group.

The colony variation in bacteria of this and other groups not being of the same character as those seen in the proteus group, are spoken of as "smooth" and "rough" abbreviated, for convenience, to "S" and "R."

As yet, however, there is lacking any clear definition of the exact meaning of "smooth" and "rough" as applied to bacterial colonies so that, in the interests of exact terminology it has been suggested that they be replaced by "matt" and "glossy."

The main differences, however, are in general as follows: the "smooth" (or glossy) colony is round, shining, translucent, reticular, and of smooth appearance, while the "rough" (or matt) colony is of an irregular or jagged contour, somewhat more opaque to transmitted light, and presents the appearance of a rough, matt surface.

Studies of these S and R forms showed no appreciable differences in their morphologic or biochemical reactions but very profound differences in other characteristics which may be summarized in tabular form as follows:<sup>5</sup>

#### SMOOTH TYPE

1. Emulsions stable in 0.85 per cent NaCl.
2. Growth uniformly turbid in broth.
3. Motile (in the case of flagellated bacteria).
4. Agglutinated by specific serum in large flocculent clumps.

#### ROUGH TYPE

1. Agglutinated by 0.85 per cent NaCl.
2. Growth forms sediment with clear supernatant fluid.
3. Motility often reduced or absent.
4. Agglutinated in small compact granules falling to the bottom of the tube.

These differences in the type of agglutination secured with S and R forms are of particular and practical importance in relation to diagnostic agglutination reactions and have been rather extensively studied, as a result of which rather definite information is accumulating.

O agglutination is granular, forms slowly, settles slowly, and presents flakes of fairly small and uniform size, leaving a clear supernatant fluid with a scanty sediment difficult to dislodge.

H agglutination, on the contrary, forms rapidly and settles rapidly, is flocculent in character, the flakes being in general large, though varying in size, and the supernatant fluid is cloudy while the sediment is voluminous and easily dislodged.

According to the observations of Weil and Felix<sup>4</sup> the agglutinins produced after inoculation with heat-killed vaccines are mainly of H type while those formed as a result of an actual attack of the disease are mainly of O type.

The practical importance of such a conclusion applied to diagnostic agglutination reactions has very naturally led to many studies.

Eldering and Larkum<sup>6</sup> from a study of 419 tests conducted with both O (alcohol-killed) and H (formalin-killed) antigen suspensions found the assumption relatively accurate, residual O agglutinins being present in quantity only after actual infection, although, of course, it must be borne in mind that the type of agglutinins following vaccination will naturally be in some measure dependent upon the preponderance of O or H antigens in the vaccine used.

Other observations<sup>7, 8</sup> are also corroboratory of these conclusions so that, while further observations and the study of large series should still be carried

on, the formulation of the following tentative dieta seems justified with regard to the diagnosis of enteric fevers by means of agglutination reactions:

1. As it has been conclusively demonstrated that both H (flagellate) and O (somatic) agglutinins are produced in most cases of typhoid fever it is advisable that agglutination tests be conducted with both H (formalin-killed) and O (alcohol-killed) antigen suspensions.

Dulaney, Wickle, and Trigg<sup>7</sup> from their studies prefer 1 per cent formalinized suspensions because the concentration inhibits the O agglutination.

2. The titer of the O agglutination is of definite value although further investigations will probably be required to determine the exact status of low titer reactions, concerning which there has been some discussion.

Felix originally suggested an agglutination titer of 1:100 or 1:200 as strongly suggestive if not diagnostic of infection. Dulaney, Wickle, and Trigg, however, finding such titers exceeded by 23 per cent of their nontyphoid cases, suggest that the titer be raised to 1:500 before being regarded as diagnostic.

On the other hand others, among them Gilbert, Coleman, and Laviano<sup>8</sup> regard agglutination in a 1:80 titer of distinct significance.

3. From the foregoing it follows that all diagnostic agglutination tests should be quantitative in character and devised to determine the highest titer in which agglutination occurs which, indeed, has long been the custom of most laboratories and should be the rule in all.

While all that has been said thus far applies to the Widal reaction in its application to the study of typhoid fever, it is important to emphasize that in principle as well as practical application it applies also to all agglutination tests or reactions.

It is essential also to recognize that the term "enteric fever" must be recognized as a generic term and as a clinical rather than a bacteriologic entity. For it is now recognized that there are at least twelve species within the *Salmonella* group which, though more commonly associated with acute gastroenteritis, are likewise capable of producing a continued fever in man. In addition to these, experience shows that in a possible explanation of continued fever in the human being both undulant fever and tularemia must be remembered.

This is of practical importance as concerns the selection of antigen suspensions against which the serum should be tested, in the determination of which several factors must be taken into consideration. In addition to the typhoid group (T, A, and B) the routine inclusion of abortus suspensions will be found of value, while the use of a *B. tularensis* suspension may either be routine, where this disease is encountered with any frequency or reserved for such cases in which this disease is regarded as a diagnostic possibility. This same principle applies also to the infrequently encountered *B. paratyphosus C* (in the United States, at least), as well as to *B. enteritidis* and the other occasional causes of enteric fever. While it is suggested above that paratyphoid A suspensions constitute one of the routine antigens, this infection is so infrequently encountered that its inclusion is a matter of choice.



From a consideration of the available evidence, and the literature on this subject is assuming quite respectable proportions, it appears that: The determination of both somatic (O) and flagellar (H) antigens is advisable; and that the determinations have their most definite value when they are quantitative.

The question is of interest not only to the laboratory worker by whom the determinations are made but as well to the clinician by whom they are requested and who, in the majority of instances, will be responsible for their interpretation and clinical application.

The interpretation of agglutination reactions is governed by many factors in addition to those already discussed.

As agglutinins in enteric fever do not reach their maximal titer as a rule before the sixteenth to the twenty-first day, although they usually begin to appear during the first week, it follows that a negative reaction during the first week is of little diagnostic significance. It is not, however, valueless because it affords a valuable base line for comparison of any future fluctuations which may occur.

While, with repeated tests, 98 per cent of typhoid fever cases show the presence of agglutinins at some time during the course of the disease, and while, when both O and H antigens are used, the absence of a significant rise in the titer is exceptional, the incidence of positive reactions on a single test is accepted as that laid down by Park and Williams, namely: first week, 20 per cent; second week, 60 per cent; third week, 80 per cent; fourth week, 90 per cent.

In the interpretation of single reactions one must also take into account the frequency and concentration of H and O agglutinins both in the serum of the normal population and in subgroups differentiated by some particular experience, such as past epidemics or antityphoid vaccination. This, of course, varies in different localities.

It appears, however, that O agglutinins in low titer (1:20 to 1:50) are more common than H agglutinins in the normal nonvaccinated population, and likewise that T.A.B. vaccination results in a higher titer of H than O agglutinins.

In general, therefore, it may be concluded that a reaction of 1:80 with an alcohol-treated (O) antigen suspension usually suggests typhoid fever or an infection with a closely allied bacterial species, while a similar reaction with a formalinized (H) antigen suspension suggests several alternatives: that the patient has typhoid fever; that he has had the disease in the past; or that he has been vaccinated.

The principles governing the interpretation of agglutination reactions in enteric fever have been well summarized by Topley<sup>10</sup> and are here paraphrased:

1. A negative reaction to all the antigen suspensions used for the test may signify: (a) that the patient does not have enteric fever; (b) that the specimen has been taken before agglutinins have appeared in the blood; or, (c) that the enteric fever is of unusual type and caused by an organism not represented in the suspensions employed in the test.

The first two alternatives are differentiated by a repetition of the test at suitable intervals; the third requires further investigation directed especially toward detection of the organism by culture.

A negative reaction in the second or third week of the illness is of definite negative diagnostic value and repeated negative reactions render the correctness of the clinical diagnosis unlikely.

2. A positive reaction against any suspension must be considered in terms of: (a) the distribution of agglutinins among the normal population; (b) the stage of the disease at which the test was made; and (c) the history as regards vaccination.

A positive reaction of 1:40 or over in a nonvaccinated individual is of definite significance and probably not an expression of "normal" agglutinins

3. As is the case with many other laboratory procedures, the trend of a series of agglutination tests is often of more value than a single reaction, especially in the case of those who have had antityphoid vaccination, any significant rise from the original titer having a decided significance.

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—R. A. K.

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## *CLINICAL AND EXPERIMENTAL*

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### GLUTATHIONE CONTENT OF BLOOD IN CHRONIC ARTHRITIS AND RHEUMATOID CONDITIONS

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THE encouraging therapeutic results obtained with parenterally given colloidal sulphur in cases of chronic nonspecific arthritis and rheumatoid conditions<sup>1</sup> prompted this investigation as a first step in the study of sulphur metabolism in these diseased states. Since the discovery of glutathione by Hopkins in 1920, much work has been centered around this interesting sulphur compound which represents a thermostabile oxidation-reduction system, and which is assumed to have some important part in the physiologic oxidation-reduction processes of the cells. Normal human blood contains from 30 to 40 mg. of glutathione per 100 c.c.; it seemed of interest to investigate whether or not possible derangements in the sulphur metabolism of our patients are expressed in abnormal glutathione values. By the method described below, the amounts of glutathione were determined in the blood of 28 healthy individuals, of 27 patients with hypertrophic arthritis, of 18 with atrophic arthritis, and of 8 with bursitis and peri-arthritis (fibrositis). At the close of this experimental work it was noted that the Arthritis Committee of the British Medical Association in a recent report recommended this subject, among others, for research in the study of arthritis.<sup>2</sup>

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## ANALYTICAL METHOD\*

For the determination of reduced glutathione in blood, Tunncliffe introduced the iodometric titration of blood filtrates obtained by deproteinization with trichloroacetic acid.<sup>3</sup> Numerous modifications of this method were evolved, mainly because iodine oxidizes under prevailing conditions not only glutathione but also ergothioneine, another substance with a sulphhydryl group always present in blood. Woodward and Fry<sup>4</sup> made the valuable observation that at high degrees of acidity and low temperature only a small fraction of ergothioneine is affected by iodine. In this laboratory it was found that the presence of a considerable excess of potassium iodide during titration further depresses the iodine consumption of ergothioneine, so that error from this source becomes negligible. The assertion of Woodward and Fry, that in the Folin-Wu method of deproteinization reduced glutathione is rapidly oxidized due to the slight acidity of the filtrate, was not substantiated. No change was found when the Folin-Wu filtrates were allowed to stand for as long as three hours; even after standing all night at room temperature the decrease in reduced glutathione was but slightly over 20 per cent. This observation agrees with that of Benedict and Gottschall<sup>5</sup> who worked with

TABLE I  
REDUCED GLUTATHIONE IN THE BLOOD OF HEALTHY INDIVIDUALS

| NAME    | SEX | AGE | CELL VOLUME<br>PER CENT | GLUTATHIONE IN 100 C.C. OF |            |
|---------|-----|-----|-------------------------|----------------------------|------------|
|         |     |     |                         | WHOLE BLOOD                | CORPUSCLES |
| B. K.   | F   | 26  | 46                      | 39                         | 84         |
| B. W.   | F   | 35  | 38                      | 33                         | 86         |
| I. D.   | F   | 26  | 40                      | 39                         | 97         |
| L. M.   | F   | 39  | 39                      | 30                         | 78         |
| M. B.   | F   | 22  | 41                      | 38                         | 94         |
| K. M.   | F   | 25  | 46                      | 40                         | 88         |
| M. S.   | F   | 22  | 43                      | 46                         | 106        |
| M. B.   | F   | 33  | 37                      | 41                         | 110        |
| A. F.   | F   | 30  | 42                      | 49                         | 118        |
| E. K.   | F   | 33  | 39                      | 39                         | 100        |
| N. J.   | F   | 28  | 43                      | 43                         | 100        |
| A. B.   | F   | 23  | 43                      | 38                         | 89         |
| C. S.   | F   | 21  | 37                      | 40                         | 107        |
| E. S.   | M   | 21  | 45                      | 37                         | 82         |
| J. T.   | M   | 25  | 44                      | 45                         | 101        |
| S. S.   | M   | 24  | 48                      | 37                         | 77         |
| A. S.   | M   | 24  | 46                      | 47                         | 101        |
| B. C.   | M   | 22  | 45                      | 37                         | 84         |
| B. D.   | M   | 25  | 48                      | 41                         | 86         |
| A. S.   | M   | 43  | 46                      | 39                         | 85         |
| C. S.   | F   | 30  | 37                      | 31                         | 85         |
| B. P.   | M   | 24  | 50                      | 41                         | 83         |
| L. K.   | M   | 20  | 52                      | 36                         | 69         |
| A. S.   | F   | 13  | 40                      | 34                         | 85         |
| B. F.   | F   | 36  | 36                      | 36                         | 100        |
| M. H.   | M   | 64  | 40                      | 45                         | 111        |
| A. N.   | M   | 51  | 44                      | 31                         | 71         |
| L. N.   | F   | 35  | 35                      | 29                         | 82         |
| Average |     |     | 42.6                    | 36.5                       | 91.3       |

\*This method was devised by Dr. Michael Somogyi under whose guidance the analytical work was carried out.

blood filtrates of about the same acidity as that of the Folin-Wu filtrates. In the iodometric titration the excellent Okuda technic<sup>6</sup> was adopted.

The analytical procedure, accordingly, has the following course: Protein-free blood filtrate is prepared by a slightly modified Folin-Wu method<sup>7</sup> in 1:10 dilution; separate the precipitate without undue delay by centrifugation, pour the protein-free fluid through a dry filter paper. Pipette 20 c.c. of the filtrate (which corresponds to 2 c.c. of blood) into an Erlenmeyer flask (100-150 c.c.) and add 1 c.c. of N/1 sulphuric acid. (At this stage the sample may be allowed to stand until a convenient time for titration.) Before titration, introduce 0.3 gm. of potassium iodide and 3 drops of 0.5 per cent starch solution (Lintner's soluble starch), cool the mixture in ice water and from a microburette titrate with 0.0025 N potassium iodate, or biniodate, until the faintest perceptible blue color becomes permanent.

$$\frac{\text{c.c. iodate used} \times 307}{8} = \text{mg. reduced glutathione per 100 c.c. of blood.}$$

All the reagents employed in this analysis keep indefinitely. One-tenth normal potassium biniodate may be prepared as stock solution and 1:40 dilutions of it made from time to time. The potassium iodide may be added in the form of 3 c.c. of a 10 per cent solution but it must not be older than one day.

The results of our experiments are presented in Tables I to V.

TABLE II  
REDUCED GLUTATHIONE IN HYPERTROPHIC ARTHRITIS

| NAME    | SEX | AGE | CELL VOLUME<br>PER CENT | GLUTATHIONE IN 100 C.C. OF |            |
|---------|-----|-----|-------------------------|----------------------------|------------|
|         |     |     |                         | WHOLE BLOOD                | CORPUSCLES |
| A. S.   | M   | 52  | 47                      | 46                         | 98         |
| P. T.   | F   | 48  | 35                      | 39                         | 111        |
| B. M.   | M   | 60  | 45                      | 44                         | 98         |
| R. G.   | F   | 58  | 36                      | 36                         | 98         |
| S. W.   | F   | 52  | 38                      | 41                         | 108        |
| C. P.   | F   | 39  | 36                      | 33                         | 92         |
| Y. E.   | F   | 54  | 40                      | 40                         | 101        |
| E. S.   | F   | 50  | 44                      | 41                         | 93         |
| S. B.   | F   | 42  | 43                      | 37                         | 85         |
| R. S.   | F   | 57  | 43                      | 41                         | 96         |
| B. W.   | F   | 41  | 36                      | 25                         | 69         |
| A. S.   | F   | 44  | 45                      | 38                         | 87         |
| W. F.   | M   | 60  | 49                      | 38                         | 78         |
| P. K.   | M   | 51  | 44                      | 41                         | 94         |
| E. L.   | F   | 53  | 42                      | 42                         | 100        |
| J. W.   | M   | 77  | 44                      | 42                         | 96         |
| R. C.   | F   | 38  | 37                      | 36                         | 99         |
| B. S.   | F   | 49  | 38                      | 34                         | 88         |
| L. H.   | F   | 56  | 38                      | 40                         | 106        |
| B. C.   | F   | 42  | 42                      | 40                         | 96         |
| E. C.   | F   | 46  | 42                      | 27                         | 65         |
| A. Z.   | F   | 57  | 41                      | 33                         | 80         |
| E. W.   | F   | 68  | 40                      | 42                         | 104        |
| D. Y.   | F   | 68  | 43                      | 40                         | 93         |
| J. B.   | F   | 51  | 40                      | 31                         | 79         |
| M. S.   | M   | 60  | 47                      | 40                         | 86         |
| L. P.   | F   | 45  | 31                      | 39                         | 123        |
| Average |     |     | 41                      | 38.1                       | 93.4       |

In Table I are recorded the glutathione determinations in the blood of 28 healthy individuals of both sexes and of varying ages, for comparison with the results obtained on patients which, grouped according to the class of disease, are given in Tables II to V.

TABLE III  
REDUCED GLUTATHIONE IN ATROPHIC ARTHRITIS

| NAME    | SEX | AGE | CELL VOLUME<br>PER CENT | GLUTATHIONE IN 100 C.C. OF |            |
|---------|-----|-----|-------------------------|----------------------------|------------|
|         |     |     |                         | WHOLE BLOOD                | CORPUSCLES |
| L. L.   | F   | 38  | 32                      | 36                         | 114        |
| M. H.   | F   | 34  | 40                      | 39                         | 96         |
| B. B.   | F   | 32  | 37                      | 40                         | 107        |
| M. W.   | F   | 47  | 37                      | 36                         | 97         |
| S. B.   | M   | 31  | 37                      | 36                         | 96         |
| M. F.   | M   | 53  | 30                      | 26                         | 86         |
| M. W.   | F   | 43  | 42                      | 35                         | 84         |
| S. H.   | F   | 45  | 39                      | 39                         | 100        |
| J. A.   | F   | 50  | 37                      | 40                         | 107        |
| R. B.   | F   | 46  | 35                      | 35                         | 100        |
| N. P.   | F   | 29  | 37                      | 35                         | 93         |
| S. B.   | F   | 45  | 36                      | 31                         | 86         |
| D. B.   | F   | 45  | 40                      | 37                         | 93         |
| R. U.   | F   | 55  | 40                      | 32                         | 81         |
| R. S.   | F   | 19  | 44                      | 32                         | 74         |
| R. K.   | F   | 20  | 37                      | 37                         | 98         |
| R. S.   | F   | 33  | 43                      | 38                         | 88         |
| G. M.   | F   | 29  | 40                      | 50                         | 124        |
| Average |     |     | 37.9                    | 36.2                       | 95.7       |

TABLE IV  
REDUCED GLUTATHIONE IN FIBROSITIS

| NAME    | SEX | AGE | CELL VOLUME<br>PER CENT | GLUTATHIONE IN 100 C.C. OF |            |
|---------|-----|-----|-------------------------|----------------------------|------------|
|         |     |     |                         | WHOLE BLOOD                | CORPUSCLES |
| M. G.   | F   | 50  | 40                      | 35                         | 88         |
| R. G.   | F   | 58  | 38                      | 35                         | 92         |
| M. L.   | F   | 31  | 41                      | 40                         | 97         |
| D. C.   | M   | 42  | 46                      | 46                         | 100        |
| A. S.   | F   | 43  | 38                      | 37                         | 98         |
| M. M.   | M   | 58  | 43                      | 38                         | 89         |
| A. U.   | F   | 61  | 36                      | 41                         | 114        |
| A. P.   | M   | 52  | 51                      | 46                         | 89         |
| Average |     |     | 41.7                    | 39.9                       | 95.9       |

TABLE V  
COMPARISON OF VARIATIONS AND AVERAGES OF THE REDUCED BLOOD GLUTATHIONE IN NORMAL INDIVIDUALS AND IN CASES OF CHRONIC ARTHRITIS AND FIBROSITIS

| TYPE OF CASES          | NUMBER<br>OF<br>CASES | CELL<br>VOLUME<br>PER CENT | AVERAGE | MG.<br>PER CENT<br>WHOLE<br>BLOOD | AVERAGE | MG. IN<br>100 C.C.<br>OF COR-<br>PUSCLES | AVERAGE |
|------------------------|-----------------------|----------------------------|---------|-----------------------------------|---------|------------------------------------------|---------|
| Normal individuals     | 28                    | 35.2 to<br>52.6            | 42.6    | 28.8 to<br>49.1                   | 36.5    | 69 to<br>118                             | 91.3    |
| Hypertrophic arthritis | 27                    | 31.5 to<br>49.0            | 41.0    | 24.9 to<br>46.1                   | 38.1    | 69 to<br>123                             | 93.4    |
| Atrophic arthritis     | 18                    | 30.0 to<br>43.8            | 37.9    | 25.7 to<br>49.7                   | 36.2    | 74 to<br>124.3                           | 95.7    |
| Fibrositis             | 8                     | 36.0 to<br>51.4            | 41.7    | 34.9 to<br>46.3                   | 39.9    | 88 to<br>114                             | 95.9    |

Since the entire glutathione content of blood is confined exclusively within the corpuscles, we have expressed the values both for 100 c.c. of whole blood and for 100 c.c. of corpuscles. Influenced by the same consideration, Gabbe<sup>8</sup> calculated the relation between glutathione and red cell count, while Woodward and Fry attempted to correlate the glutathione and the hemoglobin content. In my opinion, however, none of these forms of expression contributed added information beyond the figures given for whole blood.

The reduced glutathione content of the blood of healthy individuals, calculated for whole blood varied in these experiments between 29 and 49 mg. per 100 c.c., with an average of 36.5 mg. Woodward and Fry<sup>4</sup> obtained with their own method 34, Benedict and Gottschall<sup>5</sup> with a colorimetric procedure found 35 mg. as the average values which are in close agreement with ours.

#### CONCLUSION

The tables disclose, without need for comment, that the glutathione content in the blood of 27 patients with hypertrophic arthritis, of 18 with atrophic arthritis, and of 8 with fibrositis, compared with that in the blood of 28 normal individuals, shows no appreciable deviations either in regard to the range of variations or in the average values.

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## PNEUMOCOCCUS CHAIN FORMATION\*

ITS RELATION TO VIRULENCE IN MICE, OCCURRENCE AND DISTRIBUTION BY  
PNEUMOCOCCUS TYPES IN HUMAN RESPIRATORY INFECTIONS, AND ITS  
RELATION TO PROGNOSIS IN LOBAR PNEUMONIA

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LONG chains of pneumococci in the broth inoculated with the organisms producing the septicemia of mice, resulting from inoculations with pneumonic sputum is a striking phenomenon; its occurrence and significance were studied by us during the period from 1929 to 1933. It seemed to us that there is some correlation between the severity of the disease in patients and the finding of growth of this character in cultures of pneumococci recovered from sputum, lung juice and blood cultures. The microscopic observation of all cultures is imposed by the method of typing employed in our laboratory.

Organisms in the stained smear of the sputum cannot, without further tests, be conclusively classified as pneumococci even though they resemble pneumococci. The determination of their solubility in bile necessitates waiting for the culture.

Chain formation, or chaining of pneumococci, has been observed since 1893 when B. Issaef<sup>1</sup> and J. Washbourn<sup>2</sup> found that pneumococci formed chains when they were grown in homologous specific immune sera. Similar observations were made on *B. pyocyaneus* by Charrin and Roger (1889)<sup>3</sup> and on *B. coli* and *B. proteus* by Pfaundler.<sup>4</sup> When *B. coli* and *B. proteus* grew chainwise the organisms were club-formed, convoluted and stained unevenly, some failing to take the stain. The usual turbidity was present in cultures grown in broth with normal serum, but in the presence of specific immune sera, there was a heavy turbidity in the bottom of the tube and the broth was clear. The normal morphology and cultural characteristics of the organisms were restored by reseedling them in the usual broth.

A study of the relation of chain formation to virulence of the pneumococci in mice was undertaken. Pneumococcus Type IV (Cooper) cultures recovered from Case 4,613 were passed through mice daily for eight days. One cubic centimeter of the eighteen-hour culture from the mouse heart's blood was used. With each succeeding passage the chains became shorter; on the eighth day there were only occasional short chains of from three to five diplococci. The rest were in pairs. The first mouse lived twenty hours and the eighth mouse only seven hours.

A Type I culture isolated from Case 4,717 was passed through mice nine days before the chains completely disappeared. The first mouse lived for

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twenty-six hours, and the ninth mouse nine hours. A Type IX culture obtained from Case 1,408 was passed in the same manner for seven days. The first mouse lived thirty hours and the seventh died at the end of eleven hours.

A Type V culture isolated from Case 30,593 was passed through mice for four days. The colonies were "rough" when first isolated and remained so after the mouse passages. The culture continued to grow in chains. The culture from the heart of the fourth mouse failed to grow, though the mouse died. Because of the pressure of work the inoculation with the culture from the third mouse was not repeated and the series of inoculations continued.

A blood culture, Type VIII, isolated from Case 30,912, was streaked on agar plates to study the colonies. The edges were found to be irregular and

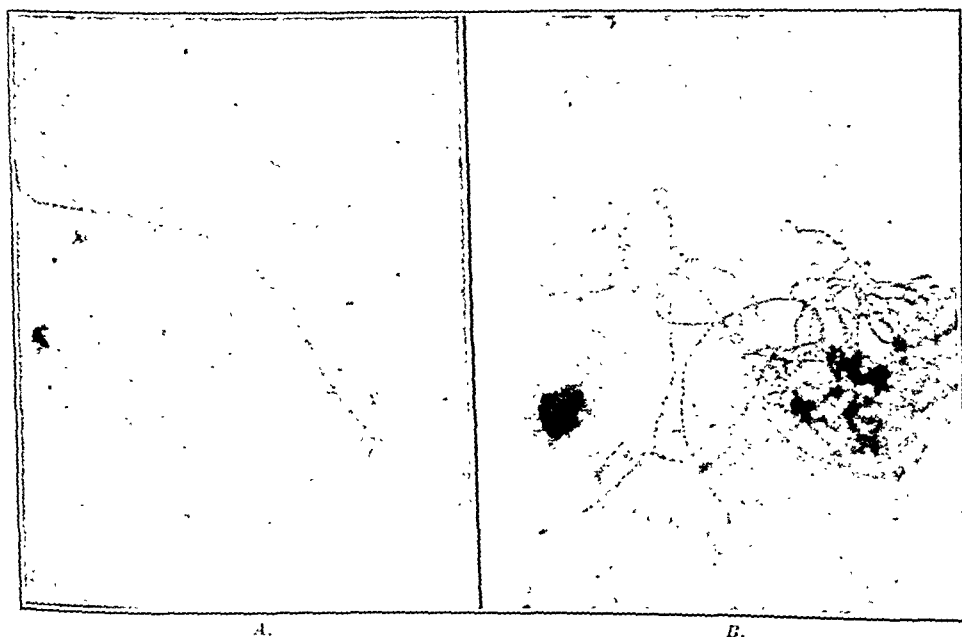


Fig. 1.—A. Pneumococci growing in chains, from mouse's heart culture. B. Agglutination with homologous serum, of pneumococci growing in chains.

the centers depressed. Broth cultures had a puff-ball growth in the bottom of the flask with some stringlike growth extending upward to the top of the broth.

A blood culture isolated from Case 21,755 gave rough colonies and the broth cultures were very granular.

Fig. 1 shows the characteristic appearance of the chain formation which extends in some instances entirely across the field with occasional separate organisms and the typical tangled skeins found in agglutination tests with pneumococci in chains. It is noteworthy that the organisms are small with ill-defined capsules.

#### CLINICAL OBSERVATIONS

Our experience seems to show that where chainwise pneumococci were present, the cases were usually less severe than the average case. During

the period of the study there were 1,152 pneumonias in adults, of whom 49 presented pneumococci in chains, an incidence of 4 per cent, and 191 pneumonias in children, of whom 7 gave pneumococci in chains, or 3 per cent.

The occurrence of the chain formation was distributed by type, as indicated in Table I. Among 145 cases from which *Pneumococcus* Type V (Cooper), Subtype IIa of Avery, were recovered, 18 strains which grew as chains were isolated, or 14 per cent, and among 76 cases of Type VIII (Cooper), atypical III of Sugg, Gaspari, Fleming and Neill,<sup>3</sup> pneumococci in chains were isolated from 6 patients, or 8 per cent. Chain formation did not occur among 163 cases of lobar pneumonia due to *Pneumococcus* Type II.

The monthly distribution of this cultural appearance during the year is shown in Fig. 2. Apparently the number of strains having chain formation



Fig. 2.—The occurrence of pneumococci growing in chains by months. The appearance of chains was most frequent when the cases were most numerous, and not in the summer time when the milder cases are more frequent. Each square is a case.

were in proportion to the number of cases, i.e., appeared most frequently when there were most cases. There were few chain-wise organisms encountered during the summer and fall months when the pneumonias are generally thought to be milder. The incidence commenced to fall when the cases were still numerous and severe in March.

It is significant that chain-wise formation was found in half the cases before the sixth day of illness. In some of the cases, our initial observation was later than that.

The only exception to the usual good prognostic correlation of the chain-wise appearance were the cases in which involvement with pneumococci of different type, which did not form chains (Case 4,915), or streptococci invasion (Case 4,808) supervened, and in two of the five bacteriemic cases.

TABLE I  
DISTRIBUTION BY TYPES OF PNEUMOCOCCUS CULTURES GROWING IN CHAINS; CULTURES ISOLATED FROM LOBAR PNEUMONIA CASES AND FROM OTHER RESPIRATORY INFECTIONS

|       |    |              |    |
|-------|----|--------------|----|
| I     | 9  | XIX          | 8  |
| II    | 0  | XX           | 1  |
| III   | 5  | XXI          | 0  |
| IV    | 5  | XXII         | 0  |
| V     | 22 | XXIII        | 1  |
| VI    | 4  | XXIV         | 0  |
| VII   | 2  | XXV          | 0  |
| VIII  | 6  | XXVII        | 0  |
| IX    | 2  | XXVIII       | 2  |
| X     | 1  | XXIX         | 1  |
| XI    | 0  | XXX          | 0  |
| XII   | 1  | XXXI         | 0  |
| XIII  | 2  | XXXII        | 0  |
| XIV   | 1  | V and VII    | 1  |
| XV    | 2  | Neg. I-XXIII | 11 |
| XVI   | 1  | Neg. I-XXXII | 1  |
| XVII  | 0  |              |    |
| XVIII | 3  | Total        | 92 |

Note the frequency of Type V and the absence of Type II.

It will be seen from the summary of diagnoses (Table II) that in only a little more than two-thirds of the cases was the lung parenchyma invaded. Four of the 92 patients died, and we shall discuss these deaths in detail later.

TABLE II  
SUMMARY OF DIAGNOSIS OF THE INFECTIONS OF PATIENTS FROM WHOM PNEUMOCOCCI IN CHAINS WERE ISOLATED

| DIAGNOSIS                         | ADULTS | CHILDREN | INFANTS | TOTAL |
|-----------------------------------|--------|----------|---------|-------|
| Upper respiratory infections      | 7      |          |         | 7     |
| Tonsillitis and peritonsillitis   | 1      | 1        | 1       | 3     |
| Sinusitis and pharyngitis         | 1      |          |         | 1     |
| Grippe                            | 1      |          |         | 1     |
| Bronchitis                        | 3      | 3        | 3       | 9     |
| Bronchopneumonia                  | 2      | 5        | 2       | 9     |
| Lobar pneumonia with tuberculosis | 2      |          |         | 2     |
| Lobar pneumonia                   | 51     | 6        | 1       | 58    |
| Pleurisy                          | 1      |          |         | 1     |
| Empyema                           | 1      |          |         | 1     |
| Total cases                       |        |          |         | 92    |

The only cases, however, in which the organisms probably responsible for death were found in chains (because there was no change of pneumococcus type) are Cases 4,612 and 25,654. In Case 4,612 the organisms were in chains in the first blood culture; in the second blood culture the pneumococci grew in chains. Subsequent blood cultures were sterile. The patient received serum, agglutinins appeared and persisted. The final increase in pulse rate may have been associated with a terminal invasion but postmortem cultures were not taken. There was very marked dehydration. Case 25,654 had a positive blood culture of pneumococci in chains on admission, Sept. 24, 1932. Subsequent blood cultures were positive on some days and negative on other days. The cultures did not grow in chains in those made after Oct. 5, 1932.



TABLE IV

A COMPARISON OF THE MORTALITY IN PNEUMONIA CASES CAUSED BY PNEUMOCOCCI OCCURRING IN CHAINS AND BY PNEUMOCOCCI NOT IN CHAINS

| CHILDREN                  |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
|---------------------------|----------------|---------------------|----------|--------|----------|-------|--------|---------------------------|----------------|----------|--------|----------|-------|--------|----------|
| PNEUMOCOCCI IN CHAINS     |                |                     |          |        |          |       |        | PNEUMOCOCCI NOT IN CHAINS |                |          |        |          |       |        |          |
| PNEUMO-<br>COCCUS<br>TYPE | TOTAL<br>CASES | TYPE<br>PER<br>CENT | NONSERUM |        |          | SERUM |        |                           | TOTAL<br>CASES | NONSERUM |        |          | SERUM |        |          |
|                           |                |                     | CASES    | DEATHS | PER CENT | CASES | DEATHS | PER CENT                  |                | CASES    | DEATHS | PER CENT | CASES | DEATHS | PER CENT |
| I                         | 1              |                     | 1        | 0      |          |       |        |                           | 49             | 24       | 1      | 4        | 25    | 2      | 8        |
| III                       | 1              |                     | 1        | 0      |          |       |        |                           | 22             | 21       | 4      | 19       | 1     | 0      | 17*      |
| IV                        |                |                     |          |        |          |       |        |                           |                | 1*       | 1*     |          |       |        |          |
| V                         | 2              |                     | 1        | 0      |          | 1     | 0      |                           | 22             | 16       | 0      |          | 6     | 0      |          |
| VI                        |                |                     |          |        |          |       |        |                           |                | 1*       | 0*     |          | 1*    | 0*     |          |
| VII                       |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| VIII                      |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| XII                       |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| XIII                      |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| XIV                       | 1              |                     | 1        | 0      |          |       |        |                           | 67             | 53       | 7      | 13       | 14    | 2      | 14       |
| XV                        |                |                     |          |        |          |       |        |                           |                | 3*       | 1*     | 33*      |       |        |          |
| XIX                       | 1              |                     | 1        | 0      |          |       |        |                           | 26             | 25       | 2      | 8        | 1     | 1      |          |
| XXVIII                    |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| XXIX                      | 1              |                     | 1        | 0      |          |       |        |                           | 5              | 5        | 1      | 20       |       |        |          |
|                           |                |                     |          |        |          |       |        |                           |                | 1*       | 1*     |          |       |        |          |
| "x"<br>group              |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| Mixed in-<br>fection      |                |                     |          |        |          |       |        |                           |                |          |        |          |       |        |          |
| Total                     | 7              | 34                  |          |        |          |       |        |                           | 191            |          |        |          |       |        |          |

\*The asterisk indicates bacteremic cases.

required infrequently. The illness seemed to be shorter than usual. The blood counts were neither high nor extremely low, with the exception of one case (2,188) in which tuberculosis was associated, where the blood count was 7,000. There were no complications with the exception of otitis media, and an empyema which was present on admission. No attempt to give a percentage of the occurrence of the various symptoms is made because too little is known concerning the occurrence of such symptoms when pneumonias are studied and divided into their separate types.

#### FATAL CASES

The patients who died are worthy of special consideration. There were four deaths in this group of cases.

CASE 4,915.—A *Pneumococcus* Type III in chains was recovered from this patient's sputum on admission, and later agglutinins for Type III were demonstrable in his blood. This patient probably acquired a Type VII pneumococcus infection from a pneumonia patient with a severe *Pneumococcus* Type VII bacteremia, who was placed in the bed next to him on the twentieth day of his illness. The temperature continued high and the patient became worse, dying on the twenty-sixth day. A Type VII pneumococcus was recovered from a lung suction culture on the day before death. Postmortem heart's blood cultures revealed a

*Streptococcus hemolyticus*. This case probably should not be counted as a death from the *Pneumococcus* Type III infection from which he had apparently recovered, having produced agglutinins, but a death from a virulent Type VII pneumococcus and a hemolytic streptococcus. It may be that a very severe clinical picture associated with a pneumococcus growing in chains should suggest that the condition possibly is due to a different organism which should be sought by further study.

CASE 4,612.—This patient had a Type V pneumococcus blood culture on admission. The organism grew in chains in the initial blood culture. The second blood culture was positive but the organisms were not in chains. All subsequent blood cultures were sterile. Marked dehydration was present throughout the illness and the patient died on the twelfth day of illness. Agglutinins for the invading organism developed and persisted. There were no pneumococci in the single sputum examination.

CASE 4,808.—Type I. This patient continued to have a high temperature, and six sputum examinations were made. In the sputum streptococci were found, as well as Type I pneumococci in chains. The results of the sputum examinations were as follows:

1. Neither pneumococci nor streptococci were recovered.
2. Streptococci.
3. No growth in mouse peritoneum.
4. Pn. Type I in chains.
5. Pn. Type I in chains.
6. Streptococcus.

This patient died on the fourteenth day and *Streptococcus hemolyticus* was recovered from the postmortem heart's blood. Two previous blood cultures taken on the third and tenth days of the illness were sterile. This patient received large amounts of *Pneumococcus* Type I serum without favorable result. The streptococci were probably of major importance, and account for the death.

CASE 25,654.—Type V. The blood cultures have been described (p. 1159). Fluid was obtained by chest tap on October 11. The patient died in pulmonary edema Oct. 13, 1932.

Seventeen of the patients received type specific serum. Seven of these were Type I, 7 Type V, 2 Type VIII, and one Type XIII. In other than Type I, experience with dosage is too limited to definitely determine the average amount required. From analogy with Type I the amounts which were efficient in all but three of the patients appear to be moderate as they were less than 50,000 units in eight instances. One hundred thousand units is our usual dose for Type I. Case 4,753, admitted on the second day, received 185,000 units of Type I serum. A gradual fall of temperature occurred on the seventh day. There had been a critical fall of pulse on the third day, and of temperature on the fourth day, the former after 25,000 units and the latter after 55,000 units. The defervescence did not last and the agglutinins did not persist in the blood. A small amount of fluid was removed. Empyema did not develop.

#### SUMMARY

1. The phenomenon of chain formation in pneumococci and its association with "rough" colonies and lowered virulence for mice is described.
2. Pneumococci growing in chains occurred in 4.2 per cent of the adult pneumococcus pneumonias, and in 3.4 per cent of the childhood pneumonias.

It occurred in 14 per cent of all Type V pneumococcus pneumonias, in 8 per cent of all Type VIII pneumococcus pneumonias and in 5 per cent of all Type XIII cases.

3. The occurrence of chain-wise growth of pneumococci in severely ill patients suggests the possibility that the infection may be due to some other organism, either a streptococcus or a pneumococcus of another type. Chain-wise appearance occurring in pneumococci recovered from the blood should not be a deterrent to the use of available specific serum as the appearance of chain formation may be due to a partial protection in the blood. This protection may become exhausted.

4. The cases for the most part appear to be less severe than pneumonias due to pneumococci which do not form chains.

The authors record their appreciation of the criticism and suggestions of Dr. William H. Park and Georgia Cooper.

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## OBSERVATIONS ON OXYGEN THERAPY\*

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WITHIN recent years oxygen therapy has become increasingly popular in clinical practice, and has proved of definite value in helping certain patients. But the question has arisen whether its more widespread use is entirely deserved. The problem can be approached in one of two ways: (1) by making a statistical summary of its effects based on clinical impression; and (2) by determining its action on the various functions of the body in controlled experiments on a few subjects, and from such determinations estimating the possible benefit in various types of illnesses. This is not the place to enter into the relative merits or limitations of the two types of approach. The recent studies on oxygen therapy with which the author was associated<sup>1, 2, 3, 4</sup> were based on the latter method, and this method of approach will be followed in this communication.

It is definitely established that oxygen is essential for the life and well-being of all body cells. Its removal or diminution is detrimental to all cells, affecting some more rapidly than others. The cells contain practically no store of oxygen and are, therefore, entirely dependent on the oxygen brought to them in the capillaries. In many diseases an interference with the oxygen supply to the tissues occurs, a condition known as anoxemia, or more accurately, anoxia. Anoxia may be localized to one region or may be general throughout the body. It may be mild, moderate, or severe in degree; it may be acute, subacute or chronic in time; and it may be one of several varieties. Barcroft<sup>5</sup> recognized three forms of anoxia: (1) The anoxic form in which the oxygen want of the tissues is due to an imperfect aeration of the arterial blood so that the blood reaching the capillaries does not carry oxygen up to its full capacity. (2) The anemic form in which the arterial blood carries its full capacity of oxygen, but the oxygen carriers are decreased in amount either because the hemoglobin is diminished in concentration or is changed in character. (3) The stagnant form, in which the arterial blood is saturated with oxygen to capacity and the oxygen carriers are adequate in amount, but the difficulty is in the slow rate at which blood passes through the capillaries. Recently Peters and Van Slyke<sup>6</sup> have added a fourth form, the histotoxic, in which the cells themselves have difficulty in utilizing the available oxygen. Of course, in many diseases two or more forms of oxygen want may be combined. This division of oxygen want into various forms and degrees is of more than academic importance, because, as will be shown in a moment, a consideration of these facts will determine the rational application of oxygen therapy.

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What can oxygen therapy do? It is quite obvious that increasing the proportion of oxygen in the air which the patient inhales from one-fifth, normally present at sea level, to one-half of the total air breathed will increase the oxygen content in his alveoli, and therefore, the quantity of oxygen passing into the blood. The amount of oxygen that can go into physical solution in the blood is very small; practically all of it combines in a loose chemical form with the hemoglobin. Obviously, when the hemoglobin is saturated with oxygen very little more can be added. It is, therefore, self-evident that when the oxygen want is of the anemic, stagnant, or histotoxic forms no improvement from oxygen therapy may be expected. In carbon monoxide poisoning, oxygen and carbon monoxide can be viewed as competitors for hemoglobin, and increasing the available oxygen will drive off the carbon monoxide more rapidly. Oxygen therapy, therefore, may be of value in this condition.

Oxygen therapy is primarily of value when the arterial blood does not contain oxygen up to its capacity. But it may be used also where it is anticipated that the arterial blood will become unsaturated because the demands of the tissues for oxygen are expected to increase. This happens especially in postoperative states. In such conditions oxygen therapy might be considered as a prophylactic measure.

Oxygen therapy may not always raise the oxygen content of the arterial blood when the blood is not fully saturated with oxygen. Let us investigate this point further. Incomplete saturation of the arterial blood with oxygen may be due to several categories of causes. One cause is a short circuit of some of the venous blood which avoids air-containing alveoli. Some of the blood returning to the arteries remains in its "venous" state. Congenital heart disease, pneumonia, and atelectasis of the lungs may give rise to these shunts. It is obvious that adding more oxygen to the air-containing alveoli will have little effect on the oxygen content of the arterial blood since the oxygen cannot come in contact with the blood that has been short circuited. It is for this reason that occasional cases of pneumonia have been reported in which the arterial unsaturation is not changed by residence in a high oxygen atmosphere.<sup>4, 7, 8</sup>

In the other types of arterial unsaturation, oxygen therapy may be expected to increase the oxygen content of the arterial blood. In the type of anoxia due to an excessively rapid flow of blood through the lung capillaries, such as may occur in hyperthyroidism, disseminated pulmonary embolism, pneumonia and severe exercise, oxygen therapy will accelerate the rate at which oxygen can enter the blood and so compensate for the rapidity of the blood flow. In the type of anoxia due to rapid shallow breathing, oxygen therapy, best combined with  $\text{CO}_2$ , will break the vicious cycle of shallow breathing which the oxygen want itself aggravates. In the type of anoxia due to the presence of excessive fluid in the lung alveolar walls and spaces, which follows passive congestion, inflammation, infarction and the irritant effect of war gases and foreign bodies in the lung, oxygen therapy enhances the force which drives oxygen into the blood, and therefore, tends to overcome the increased resistance offered to the passage of oxygen. In the type of anoxia due to

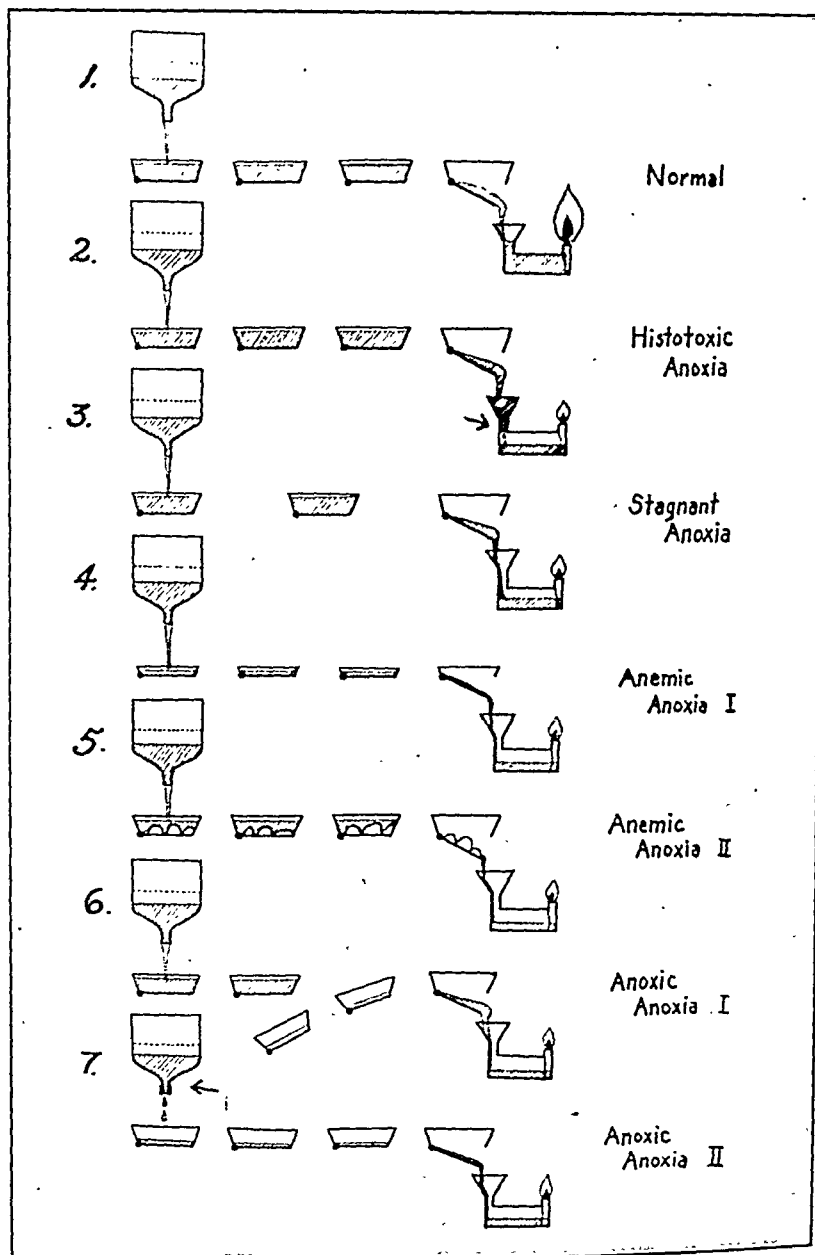


Fig. 1.—A diagrammatic representation of the processes of internal and external respiration in various types of oxygen want, which shows the mechanism of action of oxygen therapy.

In the first diagram is shown the condition normally existing; at the left, the oxygen is being poured into the blood stream, represented by little boats from the lung reservoir (external respiration). These boats, as shown by the shaded areas, are filled almost to capacity. These boats move to the right (transportation). At the right, the oxygen is shown being poured out from the blood into the tissue cells which keep the "flame of life" burning vigorously (internal respiration). Increasing the level of oxygen in the lung reservoir to the dotted line, as occurs in oxygen therapy, has little effect on the oxygen content of the boats already fully loaded.

In the second diagram is shown the condition in histotoxic oxygen want. The only difference between this condition and the normal lies in the fact that the tissue cells cannot utilize their oxygen so readily. This is indicated by a constriction in the stem of the funnel leading to the "lamp." As a consequence the "flame of life" burns less vigorously, or may even snuff out. Raising the oxygen level in the lung reservoir by oxygen therapy, can have little effect in this condition since the boats are already almost full.

obstruction of the air passages or decrease in lung expansibility, oxygen therapy will tend to compensate for the poor aeration of the alveoli.

Cyanosis has been used by clinicians as an index of a low oxygen content in the arterial blood. The recent work of Lundsgaard and Van Slyke<sup>9</sup> in particular has shown that this is an imperfect index. Cyanosis is a color change due to the presence of a definite quantity of nonoxygen carrying (reduced) hemoglobin in the capillaries beneath the skin. The reduced hemoglobin in the capillary blood is roughly the average of that in the arterial and venous blood of the region. If the circulation be sluggish, either locally or throughout the body because of heart failure or shock, then the greater removal of oxygen by the tissues will increase the reduced hemoglobin in the capillary blood above normal. If, for this reason, the reduced hemoglobin of the capillaries exceeds 5 gm. per cent, cyanosis will appear. Cyanosis, therefore, will appear more readily than normal in polycythemic individuals, and less readily than normal in the anemic. It will occur more readily than normal when the subcutaneous capillaries are engorged. It is well known among clinicians that the ashen-gray color associated with general anemia or local pallor is more significant than the slight cyanosis of the florid polycythemic individual. It will be seen from this discussion that cyanosis does not necessarily mean anoxia of the anoxic form, and, therefore, without further evidence cannot be taken as a criterion for the use of oxygen therapy.

Recent observations have shown that oxygen therapy has certain side actions which may be of value, namely, it tends to slow the rate of respiration and the minute volume of air breathed. Accompanying this there is an increase in the alkali reserve and carbon dioxide content of the blood. There is a tendency for the heart to slow (except in auricular fibrillation), and there is a drop in temperature of from  $\frac{1}{2}$  to  $1^{\circ}$  C. in patients with pneumonia according to Boothby.<sup>10</sup> The effects on respiration and the heart have been shown to occur not only in patients with oxygen want but in normal subjects as well.<sup>3, 11</sup> The manner in

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In the third diagram is shown the condition existing when the oxygen want is due to a sluggish circulation. The slow movement of blood is represented by a reduced number of boats. The delivery of oxygen to the tissue cells is decreased, hence their oxygen supply falls and again the "flame of life" decreases in intensity. Raising the oxygen level in the lung reservoir by oxygen therapy can have little effect in this condition since the boats are already almost full.

The fourth diagram represents the condition when the oxygen want is due to anemia. The small size of the boats represents the reduced capacity of the blood for carrying oxygen. The amount of oxygen reaching the tissue cells will, therefore, be decreased and the "flame" will again dwindle. Raising the oxygen level in the lung reservoir will not remedy the condition since the boats are already almost full.

The fifth diagram represents the condition when the oxygen want is due to carbon-monoxide poisoning. The reduced capacity of the blood for carrying oxygen, due to the formation of carbon-monoxide hemoglobin, is represented by the "stones" in the boats. Again the "flame of life" is lessened. Oxygen therapy, which raises the oxygen level in the lung reservoir, is of little avail except so far as it can displace carbon monoxide and dislodge some of the "stone ballast" in the boats.

The sixth diagram represents the condition when the oxygen want is due to a decreased oxygen content of the blood in the arteries, brought about by an admixture of "venous" blood. Some of the boats never pass by the lung reservoir, so that some of them are not fully laden when they reach the tissue cells. The oxygen delivery to the tissue cells is, therefore, decreased and the "flame of life" dwindles. Raising the level of oxygen in the lung reservoir will not remedy the condition because some of the partially empty boats are shunted and never reach the lung reservoir.

The last diagram shows the condition when the oxygen want is also due to a decreased oxygen content of the blood in the arteries. In this case, however, the incomplete filling of the boats is caused by an interference with the exchange of oxygen in the lungs. This is represented by a constriction at the mouth of the lung reservoir. The delivery of oxygen to the tissue cells is reduced because of the small loads carried by the boats and the "flame of life" dwindles. Obviously, increasing the level of oxygen in the lung reservoir by oxygen therapy will tend to overcome the constriction and fill the boats more completely. This is the condition in which oxygen therapy has possibilities of usefulness as discussed in the text.

which these side actions are produced is still unsolved. Whether it is some environmental factor in the oxygen chamber or a reflex set up by the increased oxygen content of the alveoli remains undetermined. However, both the slowing and diminution of respiration and the slowing of the heart are beneficial factors. The slowed heart beats more efficiently, and the load of work on the patient is decreased when respiratory effort is lessened. A similar action follows the lowering of body temperature. However, if these side actions were the only benefits to be derived from oxygen therapy they would hardly seem to warrant the trouble and expense involved in the use of oxygen.

From the foregoing analysis, it is clear that if oxygen therapy is to be used, it should be employed to increase the arterial oxygen saturation of those forms of arterial oxygen want where previous observations have shown that this can be accomplished. Oxygen therapy should be used where there is the possibility that the oxygen want contributes to the illness of the patient and where the likelihood exists that benefit will follow relief of the oxygen want. Therefore, oxygen therapy is most valuable in the acute and severe forms of oxygen want where the deleterious results of oxygen want rapidly outdistance bodily adaptations. There is less reason to use oxygen therapy in the chronic and mild forms of oxygen want where bodily adaptations have apparently compensated for the anoxia. In other words, oxygen therapy when used at all should be a temporary measure to alleviate acute, severe, generalized anoxic anoxia, where it is thought that restoring the arterial oxygen saturation will benefit the patient and check a downward clinical course. Oxygen therapy in no way acts on the underlying disease but only on the syndrome which follows anoxia. It should not be substituted for the ordinary armamentarium of the physician but be employed as an adjunct to the other forms of treatment. When given, oxygen therapy should be continuous until the desired effect is obtained, and should be maintained until the indications for its use no longer exist.

On this basis, oxygen therapy is especially valuable in postoperative conditions where rapid shallow breathing is present or where pneumonia seems imminent or has developed. It is, of course, valuable in other forms of pneumonia, but not in all cases. In cardiac patients it is valuable when there is evidence of severe passive congestion of the lungs and particularly in acute pulmonary edema. It can be of little use when these are absent and only the stagnant form of oxygen want exists. There is no evidence that it can benefit angina pectoris or long-standing cardiac edema unless these conditions are accompanied by severe pulmonary edema or stasis, except for the psychic effect on the patient which any elaborate therapeutic procedure can produce. Since it has no effect on the disease itself, oxygen therapy may fail even when the indications for its use are optimal and where relief of arterial oxygen unsaturation is attained.

The definite important place which oxygen therapy has in clinical practice will doubtless expand with further careful studies.

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## THE DIETARY TREATMENT OF UNDERNUTRITION\*

### II. EFFECT OF THE GAIN IN WEIGHT ON CARBOHYDRATE TOLERANCE

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THE use of insulin to increase the weight of individuals who are underweight, seems to us unnecessary, except for the occasional patient who lacks the appetite to eat a high calorie diet. In the first paper of this series<sup>1</sup> we showed that on a high calorie diet the gain in weight over a period of six weeks was as great as the gains reported with the use of insulin,<sup>2,3</sup> except for one case reported by Nahum and Himwich.<sup>4</sup> There are certain disadvantages to the use of insulin. Aside from the inconvenience there is the possibility of hypoglycemia. Another effect, reported by Paul, Clark, and Gibson<sup>5</sup> is that following the withdrawal of insulin in nondiabetic patients, there occurred both hyperglycemia and glycosuria. This was observed in all six of their patients. On three of the patients glucose tolerance curves were done and showed a definitely diminished tolerance for carbohydrate. In one patient, followed for eight days after the discontinuance of insulin, the tolerance had still not returned to normal.

We are reporting the effect of a high calorie diet, which was accompanied by a definite gain of weight, on the carbohydrate tolerance of the six individuals observed. The subjects were third-year medical students. The diet contained approximately 430 gm. of carbohydrate, 175 gm. of protein, and 240 gm. of fat. The total calories ingested averaged about 4,500, although as high as 5,500 was taken for the last week by Cases 7 and 8. The carbohydrate tolerance was measured by glucose tolerance curves. These were done the morning before the diet was begun and the morning after the dietary period was finished. In each case 100 gm. of glucose was given by mouth.

During the period of the diet the students continued at their work and in no way curtailed their ordinary activities. The patients in Cases 7, 8, 9,

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and 10 had always been underweight. The patients in Cases 11 and 12 had lost weight during the preceding year. The subjects were normal in all other respects.

### RESULTS AND DISCUSSION

Table I shows the weekly gain in weight in the 6 patients. The results are similar to those observed in the first group of patients.<sup>1</sup> The greatest gain in weight always occurred in the first week. The patients in Cases 11 and 12 were taking their final examinations during the last three weeks of the diet and did not eat the entire diet at this time. This is reflected in their weight charts.

TABLE I  
POUNDS GAINED WEEKLY ON DIET

| CASE    | WEEKS—POUNDS GAINED |      |      |      |     |      |     | TOTAL | WEEKS |
|---------|---------------------|------|------|------|-----|------|-----|-------|-------|
|         | 1                   | 2    | 3    | 4    | 5   | 6    | 7   |       |       |
| 7       | 5.0                 | 3.0  | 3.5  | 3.5  | 3.0 | 2.5  |     | 20.5  | 6     |
| 8       | 4.0                 | 3.75 | 3.0  | 3.25 | 2.5 | 1.75 |     | 18.25 | 6     |
| 9       | 4.75                | 2.5  | 1.5  | 1.5  | 2.0 | 2.5  | 2.5 | 17.25 | 7     |
| 10      | 5.5                 | 3.25 | 3.75 | 2.25 | 2.5 | —    |     | 17.25 | 5     |
| 11      | 7.0                 | 2.0  | 5.0  | 0.0  | 1.0 | 0    |     | 15.0  | 6     |
| 12      | 5.5                 | 0.0  | 4.0  | 1.5  | 1.5 | 0    |     | 12.5  | 6     |
| Average | 5.3                 | 2.41 | 3.46 | 2.0  | 2.0 | 1.35 |     | 16.8  |       |

TABLE II  
GLUCOSE TOLERANCE CURVES

| CASE | BEFORE DIET<br>BLOOD SUGAR MG. |       |       |       |       | POUNDS<br>WT.<br>GAINED | AFTER DIET<br>BLOOD SUGAR MG. |       |       |       |       |
|------|--------------------------------|-------|-------|-------|-------|-------------------------|-------------------------------|-------|-------|-------|-------|
|      | FAST                           | ½ HR. | 1 HR. | 2 HR. | 3 HR. |                         | FAST                          | ½ HR. | 1 HR. | 2 HR. | 3 HR. |
| 7    | 93                             | 138   | 132   | 102   | 93    | 20.5                    | 83                            | 102   | 111   | 106   | 84    |
| 8    | 101                            | 114   | 118   | 95    | 70    | 18.25                   | 89                            | 160   | 133   | 100   | 73    |
| 9    | 87                             | 152   | 154   | 116   | 78    | 17.25                   | 76                            | 105   | 133   | 117   | 81    |
| 10   | 80                             | 133   | 105   | 76    | 69    | 17.25                   | 80                            | 117   | 129   | 105   | 72    |
| 11   | 71                             | 86    | 125   | 105   | 86    | 15.0                    | 101                           | 121   | 104   |       |       |
| 12   | 76                             | 117   | 166   | 125   | 95    | 12.5                    | 75                            | 81    | 103   | 72    |       |

Table II shows the glucose tolerance curves on these subjects before and after the dietary period. These are all within normal limits. The average of the group is shown graphically in Chart 1. The second curve should be compared with the curve reported by Paul, Clark and Gibson<sup>5</sup> following the discontinuance of insulin. Individually the curves show that the height of the hyperglycemia after the dietary period was less in Cases 7, 9, 10, and 12, practically unchanged in 11. In the patient in Case 8 at the ½-, 1-, and 2-hour periods the hyperglycemia was greater than before the diet, although always within normal limits.

The students were weighed twice daily, before breakfast and after supper. Of the weight gained during the day, an average of 4 pounds was lost overnight. The true weight was that taken before breakfast. The constancy of this difference is shown graphically in Chart 2, which records the daily weights on Case 7. This is typical of the other five cases.

The ability of an individual to gain weight depends on his taking in more energy than he puts out. That this can be accomplished by means of a high calorie diet is shown in this group of subjects and in the group previously

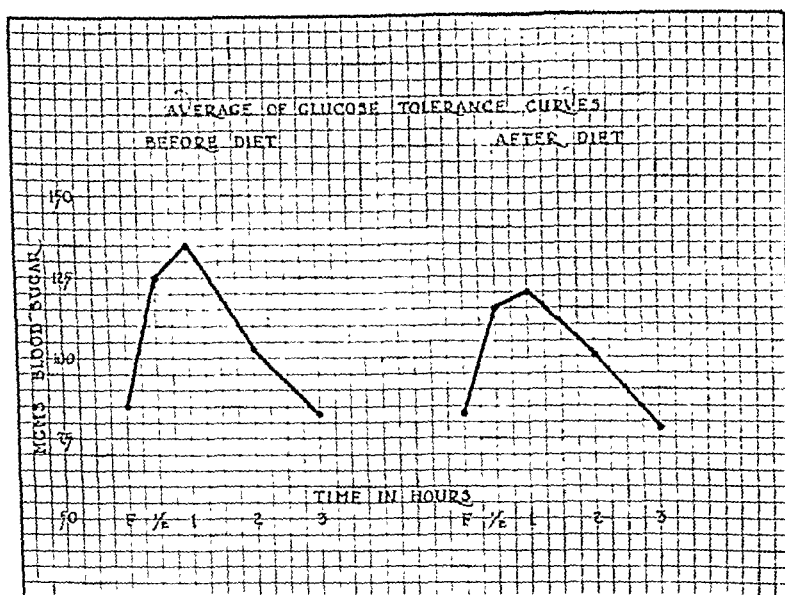


Chart 1.

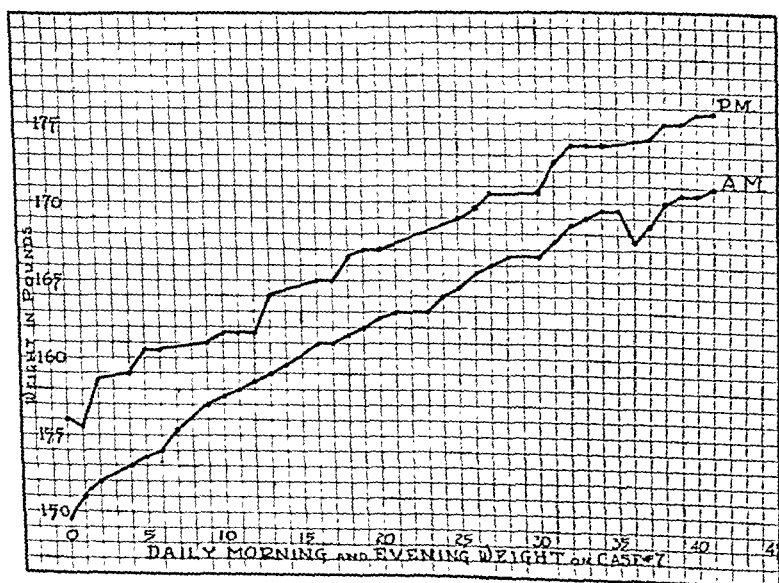


Chart 2.

reported.<sup>1</sup> The chief effect of insulin in undernutrition is its ability to stimulate the appetite. This makes it easier for the patient to eat a large amount of food. The gain in weight is due to the increased caloric intake. If the patient does not eat, insulin will not increase the weight. We had under

treatment in the hospital a patient twenty-three years of age to whom insulin was given 3 times daily before meals, ten units before breakfast, 15 before lunch, and 15 before supper. The patient had hypoglycemic reactions almost daily, but an increased appetite was not experienced. She refused to eat the entire diet and during a period of three weeks gained exactly 4 pounds.

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| HIGH CALORIE DIET   |                                         |      |      |     |
|---------------------|-----------------------------------------|------|------|-----|
| BREAKFAST           |                                         | CHO. | PRO. | FAT |
|                     | 1 glass orange juice with 2 tsps. sugar | 34   | 2    | 0   |
|                     | 2 glasses milk with 2 tsps. lactose     | 29   | 20   | 16  |
|                     | 1 oz. cereal with 2 tsps. sugar         | 33   | 3    | 0   |
| 100                 | gm. 20 per cent cream                   | 2    | 2    | 20  |
|                     | 2 slices toast                          | 36   | 6    | 0   |
|                     | 1 oz. butter                            | 0    | 0    | 25  |
|                     | 2 eggs                                  | 0    | 12   | 12  |
|                     | 2 tbs. jam or jelly                     | 25   | 0    | 0   |
| NOON                |                                         |      |      |     |
|                     | 5 oz. meat                              | 0    | 30   | 30  |
|                     | 5 oz. potato                            | 30   | 3    | 0   |
|                     | 2 fresh vegs.                           | 12   | 2    | 0   |
|                     | 2 glasses milk with 2 tsps. lactose     | 29   | 20   | 16  |
|                     | 2 slices toast                          | 36   | 6    | 0   |
|                     | 1 oz. butter (or 2 tbs. mayonnaise)     | 0    | 0    | 25  |
| 100                 | gm. dessert with 2 tbs. cream whipped   | 20   | 7    | 10  |
| NIGHT               |                                         |      |      |     |
|                     | 1 glass tomato juice                    | 5    | 1    | 0   |
|                     | 5 oz. meat                              | 0    | 30   | 30  |
|                     | 5 oz. potato                            | 30   | 3    | 0   |
|                     | 2 fresh vegs.                           | 12   | 2    | 0   |
|                     | 1 glass milk                            | 12   | 10   | 8   |
|                     | 1 slice toast                           | 18   | 3    | 0   |
|                     | 1 oz. butter                            | 0    | 0    | 25  |
|                     | 1 baked apple with 2 tsps. sugar        | 25   | 3    | 0   |
| 100                 | gm. (20 per cent) cream                 | 2    | 2    | 20  |
| NIGHT FEEDING       |                                         |      |      |     |
|                     | 1 glass milk                            | 12   | 10   | 8   |
|                     | 2 Unceda biscuits                       | 10   | 1    | 1   |
| Caloric value 4,574 |                                         | 423  | 177  | 246 |

When one considers that an impaired tolerance for carbohydrate may follow the use of insulin in nondiabetic individuals, and since a satisfactory gain of weight will follow the ingestion of a high calorie diet, it seems to us that the indiscriminate use of insulin in undernutrition is inadvisable.

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## GAUGING THE DOSE OF INSULIN

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SINCE its introduction in 1922, insulin, one of the few specifics in medicine, has found wide acceptance. Unlike other specifics (morphine, quinine, the arsenicals, etc.), there is no approximately "standard dose" for insulin. Necessarily the insulin requirement varies with the individual patient, depending upon the indeterminate insulin secretion within his own body and the glucose equivalent of his diet. Hitherto achievement of adequate insulin dosage has been a tedious process. One of the standard usages defines that the patient be placed upon an almost starvation diet until the urine is sugar-free, that the food intake be gradually increased, and finally that he be given a number of units of insulin equal to one-half the amount of glucose, measured in grams, in the twenty-four-hour urine. As the diet is further increased insulin is further readjusted depending upon the urinary glucose content. If the patient be in diabetic coma, a standard plan calls for the administration of a definite number of units of insulin, usually between 20 and 40, each hour until the urine is sugar-free.

At best the methods are cumbersome. They fail to take into sufficient account the varying renal threshold for glucose.<sup>1</sup> Not infrequently the patient shows no glycosuria although the blood sugar is well above 250 mg. per cent. On the other hand an insulin reaction may be precipitated by failure to consider residual sugar in the bladder urine; or by failure to remember that glycosuria usually continues below the glycaemic level at which it began.

The ideal treatment of diabetes is that which insures a twenty-four-hour blood sugar curve analogous to that found in the nondiabetic. In addition to restoring the disturbed osmotic balance of the blood upset by the increase in blood sugar, maintenance of a normal blood sugar curve theoretically insures complete utilization of all available carbohydrate. It is common knowledge that the continuously aglycosuric diabetic with a normal blood sugar level develops a spontaneous increase in tolerance not shown by the intermittently glycosuric diabetic. Since bodily resistance is at least as great in the adequately controlled diabetic as in the nondiabetic, intercurrent infection and subsequent debility are rare.

With these considerations in mind I have found the following empiric method of gauging the insulin requirement of individual patients useful. It must be mentioned that the method has no logical basis in fact, unless it be that clinical experience has amply confirmed its complete safety and relative value. It is assumed that all of the glucose present in the body is equally distributed in all the tissues (regardless of the possible actuality); if the

blood of a 70 kg. patient contains 500 mg. per cent glucose (0.5 gm. per 100 c.c.), then the total glucose in the entire body may be assumed to be represented by the formula

$$\frac{70,000 \text{ (gm. body weight)} \times 0.5}{100} = 350 \text{ gm.}^2$$

Since the normal blood glucose (and body tissue glucose?) is roughly 100 mg. per cent (0.1 gm.), modification of the formula enables determination of the glucose present as excess:

$$\frac{70,000 \times 0.5 - 0.1}{100} = 280 \text{ gm. representing the amount of glucose which}$$

must be either burned, excreted, or synthesized into glycogen to restore a normal blood sugar level! A number of units of insulin equal to one-half the excess glucose in grams is assumed to be required to utilize the excess glucose; as a safety measure only four-fifths the estimated dose is given.

Hence in the hypothetical case here cited, 112 units  $\left(\frac{280}{2} \times \frac{4}{5}\right)$  of insulin may be given the patient.

The formula may be simplified for routine use as follows:

$$\frac{\text{Body weight in pounds}}{6} = \text{the number of units of insulin to be given}$$

for each 100 mg. per cent the blood sugar is above the normal glycemic level (100 mg. per cent). Applying this formula to the 154-pound (70 kg.) patient

with a blood sugar of 500 mg. per cent, it is evident that 104 units  $\left(\frac{154}{6} \times 4\right)$

of insulin will be required. As a matter of actual fact it has been my usual experience that such an amount of insulin will reduce the blood sugar only four-fifths (the safety factor) of the expected reduction; however, such a procedure enables avoidance of an insulin reaction. It has been found by experience as well as by unpublished studies that this change may usually be expected to take place within from two to four hours.

#### EXAMPLES

CASE 1.—L. H., female, aged fifty, weight 154 pounds. Blood sugar at time of injection of 70 units of insulin: 300 mg. per cent. Calculated blood sugar reduction: about 200 mg. per cent. Blood sugar two hours after insulin injection: 100 mg. per cent. Actual blood sugar reduction: 200 mg. per cent.

CASE 2.—F. E., female, aged fifty, weight 176 pounds. Blood sugar at time of injection of 120 units of insulin: 471 mg. per cent. Calculated reduction: about 300 mg. per cent. Blood sugar two hours after insulin injection: 131 mg. per cent. Actual reduction 340 mg. per cent.

CASE 3.—J. D., male, aged thirteen, weight 108 pounds. Blood sugar at time of injection of 120 units of insulin: 1,000 mg. per cent. Blood sugar two hours later: 550 mg. per cent. An additional 80 units of insulin was given. Calculated total reduction: about 820 mg. per cent. Blood sugar two hours after second insulin injection: 133 mg. per cent. Actual reduction: 867 mg. per cent. Some hours later the patient had to be given, at intervals, two small doses of adrenalin for the relief of clinical insulin shock. The following morning, sixteen hours after the last blood analysis, the blood sugar was 200 mg. per cent. The

importance of prompt and energetic treatment in diabetic coma is emphasized by the finding of a blood nonprotein nitrogen at this time of 42 mg. per cent; one may only conjecture how high it may have been during the intense acidosis accompanying the very high blood sugar. In another patient treated according to the same principles, the blood nonprotein nitrogen fell from 75 mg. per cent to 64 mg. per cent in ten hours and to 45 mg. per cent in three days; during the first ten hours the blood sugar fell from 900 mg. per cent to the normal level.

CASE 4.—L. M., female, aged fifty-three, profound coma, estimated weight: 75 or 80 pounds. Given 50 units of insulin on admission to the hospital. Blood sugar just before injection: 1,100 mg. per cent; blood nonprotein nitrogen: 119 mg. per cent. Complicating bronchopneumonia. As soon as the blood sugar report was obtained, the patient was given an additional 100 units of insulin. Calculated total reduction: about 830 mg. per cent. It was not possible to obtain another blood sugar determination until ten hours after the second insulin injection; at that time the blood sugar was 117 mg. per cent, the nonprotein nitrogen, 200 mg. per cent. Actual blood sugar reduction: 983 mg. per cent. Subsequent autopsy revealed an extensive nephrotic process in both kidneys as well as the bronchopneumonia noted at entry to the hospital.

*Comment:* The body weight formula described above (last formula) would have called for 160 units of insulin in Case 3 instead of the 200 actually given, and in Case 4 for 130 units instead of 150. Hence, it is felt that the margin of safety allowed by the revised formula is sufficiently great to permit its employment by the general practitioner. No generalized urticarial eruption or other anaphylactic manifestation was observed after the administration of these large amounts of insulin. Several years ago the former usually followed the administration of as much as 100 units of insulin.

*Note:* Patients are carefully watched for the appearance of symptoms of insulin shock; if glucose or orange juice cannot be given by mouth adrenalin hydrochloride (4 to 10 minims) hypodermically may be depended upon to relieve such symptoms without waiting for the report of blood sugar analysis. If necessary it may be followed by the intravenous or intracardiac injection of 50 per cent glucose (10 to 20 c.c.).

*Note:* In management of the postoperative diabetic the blood sugar is determined two hours after the patient's return from the operating room; the insulin requirement is calculated as above and further modified as food is allowed. By the use of this method we have successfully carried one patient with a previously uncontrolled diabetes through an immediate laparotomy for acute suppurative appendicitis. Neither acidosis nor insulin reactions marred a smooth postoperative course; no intravenous glucose was necessary at any time.

*Contraindications:* Patients with known myocardial damage. Since we give no insulin by preference, a very little if absolutely required, to such patients, untoward reactions have not been observed.

#### INSULIN DOSAGE IN ROUTINE DIABETIC MANAGEMENT

If diet alone proves insufficient to render the patient aglycosuric and the blood sugar normal two hours after a meal (preferably breakfast), the patient is placed in the hospital and immediately given the diet calculated as necessary for his maintenance upon discharge. From the blood sugar deter-

mined at entry the insulin required for the metabolism of the endogenous glucose is determined according to the formula described above. Further, it is assumed that one unit of insulin will metabolize four grams of exogenous glucose;<sup>3</sup> in the same manner as before only four-fifths the estimated dose is given to allow a safety factor; this amount may be easily ascertained by giving a number of units of insulin equal to one-fifth of the available glucose in the diet as measured in grams. Exogenous available glucose is calculated in the usual manner: 100 per cent of the carbohydrate plus 60 per cent (actually only 58 per cent) of the protein plus 10 per cent of the fat. The insulin is divided into one, two, or three doses depending upon the total requirement; the largest dose is given before breakfast, the smallest before lunch, and the intermediate dose before supper. Two hours after breakfast on the third day of this regime the blood sugar is again determined to permit further insulin regulation in preparation of the patient for discharge from the hospital; usually it is between 90 and 150 mg. per cent. If at the former level, insulin dosage is reduced; if higher than 150 mg. per cent, insulin dosage is readjusted on the basis of the endogenous glucose reflected in the blood sugar level. Ordinarily the insulin readjustment made at this time is sufficient to carry the patient for several weeks until mild insulin reactions indicate the need for further readjustment. This method permits the patient to receive intensive diabetic instruction in the hospital and return to his gainful occupation in from four to seven days.

Essentially the same plan is used in the after-care of the coma patient except that instead of basing the insulin dosage for the endogenous glucose on the blood sugar either on admission to the hospital or after its reduction by a massive dose of insulin, it is arbitrarily assumed that the endogenous glucose excess is represented by a blood sugar of 300 mg. per cent.

If for any reason the diet formula needs to be changed (for instance if the carbohydrate in the diet is to be increased in preparation for operation) insulin is readjusted according to the difference in the exogenous glucose supplied by the two diets.

#### EXAMPLES

CASE 5.—M. R., female, aged thirteen, weight 65 pounds. Blood sugar on admission to the hospital: 400 mg. per cent; insulin required for metabolism of the endogenous glucose as calculated: 33 units. Insulin required for metabolism of exogenous glucose supplied by diet of protein 40 gm., fat 60 gm., carbohydrate 100 gm., available glucose 138 gm.: 28 units. Total insulin requirement: 61 units. Sixty units of insulin, 30 before breakfast, 10 before lunch, and 20 before supper were administered. Two hours after breakfast on the second day of this regime the blood sugar was 181 mg. per cent. The insulin was increased by 20 units instead of by the calculated 10 units and there were questionable mild insulin reactions. On the fifth day of this regime the diet was changed to protein 40 gm., fat 75 gm., carbohydrate 80 gm., available glucose 112 gm., a decrease of 26 gm. of available glucose calling for a reduction of 8 units of insulin (when small amounts of insulin only are involved the customary four-fifths reduction is usually omitted). On the third day of the new regime during which she received 30 units of insulin before breakfast, 15 before lunch, and 25 before supper, the blood sugar two hours after breakfast was 154 mg. per cent. During the course of the next few days mild insulin reactions indicating a considerable increase in tolerance occurred and the insulin was further reduced to 25 units before breakfast, 10 before lunch and 20 before supper. Two months after discharge from the hospital this patient

had gained 20 pounds in weight and had been able to reduce the insulin to 20 units before breakfast, none before lunch and 15 before supper. Nine months after discharge she had gained an additional 10 pounds in weight and had further reduced the insulin to 15 units before breakfast and supper.

CASE 6.—C. R., female, aged eleven, weight 62 pounds. Admitted to the hospital in coma with a blood sugar of 666 mg. per cent. Treatment seemed ineffective and on the second hospital day I was asked to see the patient. She was semistuporous and quite weak; there was no fever or infection. At that time her blood sugar was 375 mg. per cent; insulin required for metabolism of the endogenous glucose: 27 units. I changed the diet of protein 35 gm., fat 50 gm., carbohydrate 80 gm., available glucose 106 gm. with 15 units of insulin before breakfast, 10 before lunch, and 15 before supper to protein 35 gm., fat 90 gm., carbohydrate 80 gm., available glucose 110 gm. Hence no change in the insulin required for metabolism of endogenous glucose was indicated. Uncertain of the "hangover" effect of certain questionable treatment earlier employed, the insulin was increased only 15 units, a little more than half of that calculated, so that the patient now received 20 units before breakfast, 15 before lunch and 20 before supper. On the third morning of this regime the blood sugar two hours after breakfast was 138 mg. per cent and on the seventh morning at the same time it was 125 mg. per cent.

CASE 7.—E. R., male, aged thirty-two, weight 128 pounds. Blood sugar on admission: 500 mg. per cent; calculated insulin requirement: 84 units. Actually he was given 100 units and suffered an insulin reaction so mild that only one ounce of orange juice was required to relieve it. Unfortunately it was not possible to obtain a blood sugar determination until twelve hours after the insulin administration; at that time it was 140 mg. per cent. Assuming that the blood sugar would rise to about 300 mg. per cent spontaneously, 42 units of insulin would be required for its control. The diet of protein 70 gm., fat 160 gm., carbohydrate 80 gm., available glucose 138 gm., required 28 units of insulin for metabolism of the exogenous glucose. The total insulin of 70 units was given, 30 units before breakfast, 15 before lunch and 25 before supper. (To simplify the problem two intermediate days with a relatively higher carbohydrate allowance have been omitted from the discussion.) On the third day of the regime outlined above the fasting blood sugar was 190 mg. per cent while two hours after breakfast it was 125 mg. per cent. The latter figure indicates that the patient was adequately controlled; to lower the fasting blood sugar it would have been necessary to give a midnight dose of insulin, a procedure which we dislike because of the inconvenience caused the patient. The patient was discharged from the hospital on the seventh day. Three months after discharge he had gained 15 pounds in weight and reduced his insulin by 5 units, to 30 before breakfast, 15 before lunch, and 20 before supper.

*Note:* In patients running an irregular fever less than the total optimal dose of insulin as calculated is given in order to minimize the possibility of an insulin reaction when the fever reaches a low point. However, in the case of an infection with a continued fever more insulin is usually required. I am unable to propose any definite rules for the diabetic management of such patients inasmuch as their insulin requirement varies from hour to hour.

*Contraindications:* As above mentioned. In patients with severe myocardial damage no insulin is given even though the blood sugar be as high as 300 mg. per cent. If acidosis supervenes insulin may be given cautiously in doses of 5 or 10 units at a time. Onset of anginal pain is considered an absolute contraindication to its further use.

#### SUMMARY AND CONCLUSIONS

1. The blood sugar of a diabetic patient in coma or of one presenting a high blood sugar may be safely and quickly reduced by giving a number of

units of insulin equal to one-sixth the body weight in pounds for each 100 mg. per cent the blood sugar is above the normal glycemic level (100 mg. per cent). If the physician cares to use larger immediate doses of insulin, carefully watching the patient at the time, he may give one unit of insulin per kilogram body weight for each 200 mg. per cent that it is desired to lower the blood sugar. While the ordinary supportive measures such as saline hypodermoclysis and cardiac stimulants are given according to indications, glucose, sodium lactate, etc., are not ordinarily employed in the treatment of the coma patient. The rapidity with which the symptoms may be controlled makes the latter medication superfluous.

2. Routine regulation of the diabetic patient presenting no complications may be quickly and safely attained by giving a number of units of insulin determined as above for the metabolism of the endogenous glucose plus a number of units of insulin equal to one-fifth of the available glucose in the diet as measured in grams. A blood sugar two hours after breakfast on the third day of this regime determines further insulin readjustment. This method permits intensive hospital diabetic instruction and yet allows return of the patient to his gainful occupation in from four to seven days.

3. Since the formula here presented is entirely theoretical in its conception and derives its only merit from satisfactory and apparently safe clinical experience, the fact must never be overlooked that it should be modified to the need of the individual patient as determined by that patient's clinical response. It should not be used in the treatment of patients with myocardial damage.

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METROPOLITAN BUILDING

## SODIUM TAUROCHOLATE AND THE VIRULENCE OF HUMAN TUBERCLE BACILLI\*

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DURING the course of studies attempting to disclose the factors accounting for the difference in gross colony characteristics of human and bovine tubercle bacilli noted on different culture mediums, particularly the striking difference on inspissated egg mediums (low spreading wrinkled form) and on potato mediums (elevated, heavy, limited form) as well as on inspissated whole blood mediums (thin veil-like spread growth<sup>1, 2</sup>), certain substances were incorporated in different nutrient mediums for the purpose of changing the physical-chemical properties of the medium, particularly to change the surface tension of the medium. Among those tried, one, sodium taurocholate in certain concentrations, produced decided changes in the nature of the resultant growth which led to a further study of the colonies and of the bacilli. Although our studies did not disclose the exact nature of the factors determining the characteristics of the gross growth on the various mediums, it did appear that by reducing the surface tension of these mediums to a common level there grew on these different mediums identical colonies of the so-called smooth-greasy type recorded as seen predominantly with virulent avian tubercle bacillus cultures on egg mediums.<sup>3, 4</sup>

The experiments were conducted with graded plantings from one milligram to one-millionth of a milligram and varying amounts of sodium taurocholate (Eastman Kodak Co.) were added to defibrinated cow blood, to egg white, to egg yolk, and to potato cylinder mediums which were then sterilized by inspissation or autoclaving as usual. All the mediums contained 3 per cent glycerol as is customary for growing human tubercle bacilli. With defibrinated cow blood, 1 per cent of sodium taurocholate inhibited the growth of small plantings; 2 per cent inhibited medium grade plantings; and 5 per cent inhibited moderately heavy plantings. With egg white, a poorer nutrient, the inhibition effects on growth were even more striking, and consequently the effect on the colonies was inappreciable. With inspissated egg yolk and potato cylinder mediums, inhibition was noted in the smaller plantings when the mediums contain 5 per cent sodium taurocholate but not when containing 2 per cent. Most striking, however, was the effect of the 5 per cent sodium taurocholate upon heavy plantings (one milligram or more per cubic centimeter seeding) in producing smooth, solitary, greasy appearing "avian-like" colonies on the egg yolk and potato cylinder mediums.

These colonies were, therefore, studied for their characteristics, regarding particularly the virulence of the bacilli and the permanency of the changes

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produced. The former (the virulence) was studied by graded inoculation of fine suspensions into guinea pigs, a quantitative method of virulence determination, and the latter (culture characteristics) was studied by transfer back

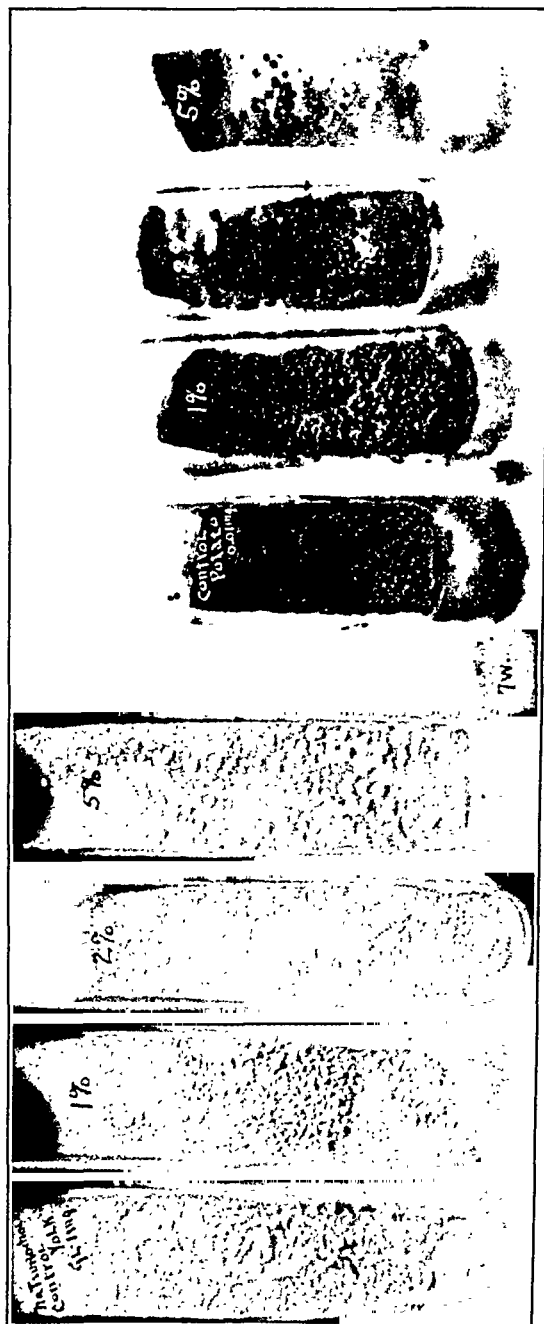


Fig. 1.—The effect of sodium taurocholate in egg yolk and potato cylinder mediums on the nature of the growth of human tubercle bacilli. The four tubes on the left are inspissated egg yolk (1 mg. per c.c. plantings), the control being on the extreme left and in order the next three tubes contain 1, 2, and 5 per cent sodium taurocholate, respectively. The remaining tubes on the right (0.01 mg. per c.c. plantings) are potato cylinder medium, the first of this set being the control and the last three containing 1, 2, and 5 per cent sodium taurocholate. The strain of virulent human tubercle bacilli pictured is strain "Glueckson", and the period of incubation seven weeks. Note the characteristic culture type of growth for egg and potato, and the change to the smooth, moist appearing globular colonies in the presence of 5 per cent sodium taurocholate.

and forth for several generations on both the inspissated egg yolk medium and the potato cylinder mediums with and without the sodium taurocholate (5 per cent).



A number of different strains of human tubercle bacilli were studied; most of them that had been cultivated on the laboratory mediums proved readily adapted to the culture tests, but a few recently isolated strains could not be grown on the 5 per cent sodium taurocholate mediums. The property of producing the solitary "so-called" smooth, greasy appearing colony was readily attained with both virulent and avirulent human tubercle bacilli on one transfer to egg or potato mediums, containing 5 per cent sodium taurocholate, and return to their original culture form was also attained by one transfer to the egg yolk or potato medium not containing the 5 per cent sodium taurocholate. This is well illustrated in the accompanying picture. The effect of the change wrought in the cultures by the sodium taurocholate on the virulence was studied by comparing the quantitative general virulence

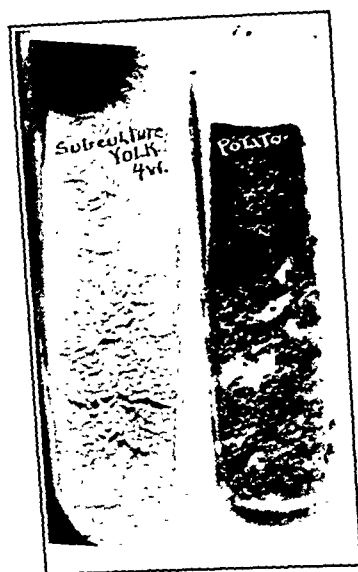


Fig. 2.—The immediate change to the characteristic growth for egg and potato after several transfers on egg or potato mediums containing 5 per cent sodium taurocholate when sub-cultured on egg yolk medium (left tube) or potato cylinder medium (right tube) without the sodium taurocholate. The strain of human tubercle bacilli is "Gluckson" and the incubation period four weeks.

of the taurocholate cultures with the ordinary cultures of the same strain, and using for this purpose both virulent and avirulent human tubercle bacilli.

The method of testing virulence, used for years in our laboratory for human and bovine bacilli, was based on the findings of one of us<sup>2</sup> from an extensive study of the effect upon the development of general tuberculosis in the guinea pig following various routes of infection. At that time it was found that the development of disease in the guinea pig differed little for practical purposes with the average strain of human or bovine bacilli, regardless of whether injected subcutaneously, intraperitoneally, or intravenously; and therefore, the subcutaneous method was chosen because of the ease of noting the development of disease by the enlargement of the local gland. The subcutaneous method has been used for general systemic virulence tests in guinea pigs and has proved of service in determining the virulence of human

and bovine tubercle bacilli with graded amounts of fine suspensions of these bacilli. The dilutions used are noted from Table I and duplicate tests (2 guinea pigs) were inoculated for each dilution being tested.

TABLE I

THE VIRULENCE OF A VIRULENT AND AN AVIRULENT STRAIN OF HUMAN TUBERCLE BACILLI AFTER GROWTH ON INSPISSATED EGG YOLK GLYCEROL-WATER MEDIUM AND INSPISSATED EGG YOLK GLYCEROL WATER 5 PER CENT SODIUM TAUROCHOLATE MEDIUM

| STRAIN      | MEDIUM GROWN ON                                       | AMOUNTS OF SUSPENSIONS IN MG. INJECTED SUBCUTANEOUSLY IN GUINEA PIGS |   |      |                  |                  |                  |
|-------------|-------------------------------------------------------|----------------------------------------------------------------------|---|------|------------------|------------------|------------------|
|             |                                                       | 10                                                                   | 1 | 0.01 | 10 <sup>-4</sup> | 10 <sup>-6</sup> | 10 <sup>-8</sup> |
| "Gluckson"* | Inspissated yolk<br>Glycerol water                    |                                                                      |   | +    | +                | +                | 0                |
| "Gluckson"  | Inspissated yolk<br>Glycerol water<br>5% Sodium Taur. |                                                                      |   | +    | +                | +                | +                |
| "Human"*    | Inspissated yolk<br>Glycerol water                    | 0                                                                    | 0 | 0    |                  |                  |                  |
| "Human"†    | Inspissated yolk<br>Glycerol water<br>5% Sodium Taur. | 0                                                                    | 0 | 0    |                  |                  |                  |

\*For a report on the Virulence of these two strains see: Corper, H. J., and Cohn. Maurice L.: The Viability and Virulence of Twelve Year Old Broth Cultures of Tubercle Bacilli Maintained at Incubator Temperature. *Am. Rev. Tuberc.* 28: 856, 1933.

†The result of examination of the guinea pigs at postmortem is recorded as + indicating a definite tuberculosis or 0 indicating the absence of macroscopic tuberculosis in any of the organs or tissues.

‡The bacilli used were taken from the third subculture on the indicated medium.

The results recorded in Table I indicate that although sodium taurocholate in 5 per cent concentration in inspissated egg yolk mediums (or potato cylinder medium, results not tabulated) causes an immediate change in the gross colony appearance to that of a smooth-greasy "avian-like" colony, but it does not enhance the virulence of avirulent human tubercle bacilli in spite of at least three continuous transfers on the taurocholate medium. A virulent strain of human tubercle bacilli would appear to have increased in virulence from the results obtained, but when analyzing these results they prove to be more apparent than real, in that a transfer back to a medium not containing taurocholate reveals the change to be rather one of temporary nature and not one of virulence increase. There are two factors which may contribute to this apparent change, one the increased concentration of the bacilli in the taurocholate colony, and the other the greater ease of preparing finer dispersed and more stable suspensions of the bacilli in the presence of the taurocholate.

In view of our findings with sodium taurocholate recorded above, and other observations not recorded here, we feel that the problem of correlation between rough colonies and avirulence, and smooth colonies and virulence for human and bovine tubercle bacilli merit further exhaustive investigation with careful control both as to virulence tests and the physical-chemical nature of the mediums. It would appear also that the characteristic of rough or smooth for human and bovine tubercle bacilli may represent changes dependent more or less upon the physical-chemical properties of the medium in close proximity to the individual colony.

## SUMMARY

1. Virulent and avirulent human tubercle bacilli grown on an inspissated egg yolk medium or on a potato cylinder medium containing 5 per cent sodium taurocholate following heavy plantings produce smooth-greasy appearing "avian-like" colony growths which can be changed by a single transplant on the same mediums not containing taurocholate.

2. Avirulent human tubercle bacilli grown through several generations on egg or potato nutrient mediums containing 5 per cent sodium taurocholate do not show an increased virulence for guinea pigs in spite of the temporary physical change in the gross colonies.

3. Virulent human tubercle bacilli grown on egg or potato nutrient mediums containing 5 per cent sodium taurocholate show a transient apparent increase in virulence for guinea pigs.

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## VII. AUTONOMIC IMBALANCE IN ANIMALS\*

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DURING the last four years we have used several hundred animals in studying the qualitative and quantitative effects of drugs affecting the autonomic system (we use the term "autonomic system" to include the central and peripheral sympathetic and parasympathetic nerve fibers, as well as the individual metabolites affecting them). We were impressed with the striking variations in the reaction of animals to epinephrine, which is considered a typical sympathicotropic stimulant, and the reaction following the administration of Desympatone† (active principle of Tissue Extract 568), which, as we have shown, stimulates the parasympathetic system.<sup>2, 4, 5, 6, 7</sup>

We were able to subdivide our animals (mostly dogs) into three groups or classes.

*Group I.*—Animals which are very susceptible to epinephrine, and relatively insensitive to desympatone.

*Group II.*—Those showing reverse phenomena, being particularly susceptible to desympatone and relatively insensitive to epinephrine.

*Group III.*—Animals showing rather a marked susceptibility to the effects of both types of drugs. In our experience we have not found a dog which failed to respond to the administration of therapeutic doses of both substances, although it is conceivable that such animals might be encountered.

This marked variation in susceptibility was emphatically called to our attention when, during one working day, tests were made upon two nonpregnant dogs weighing approximately 10 kilograms each, and anesthetized with morphine and chlorotone. Fig. 1 shows the comparative effects of epinephrine and desympatone (Dog 301). This animal showed a slight reaction to desympatone, but was extremely sensitive to epinephrine. After administering 20 gamma ( $\frac{20}{1000}$  milligram) of epinephrine, a marked rise in blood pressure was produced (Fig. 1, A). Simultaneous electrocardiograms showed dropped ventricular beats. This occurred at the height of the blood pressure rise. It is usually accepted that epinephrine causes an increase in the cardiac rate. This animal definitely showed a normal auricular rate, but the ventricular rate was cut down markedly on account of the ventricular asystoles (Fig. 2, A). In this tracing, reading from left to right, one sees an auricular complex marked P; this is followed by QRS T-waves, which represent systole of the ventricles. The QRS complex is registered by very fine lines, requiring careful examination or the use of a magnifying glass for the untrained eye. Another normal P, QRS, T follows; then three P-waves representing three auricular systoles with

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†Different lots of tissue extract contain varying small amounts of histamine and choline compounds. Chemical studies are being made to remove them. The desirable clinical effects are attributed to "Desympatone," which is a distinct entity.

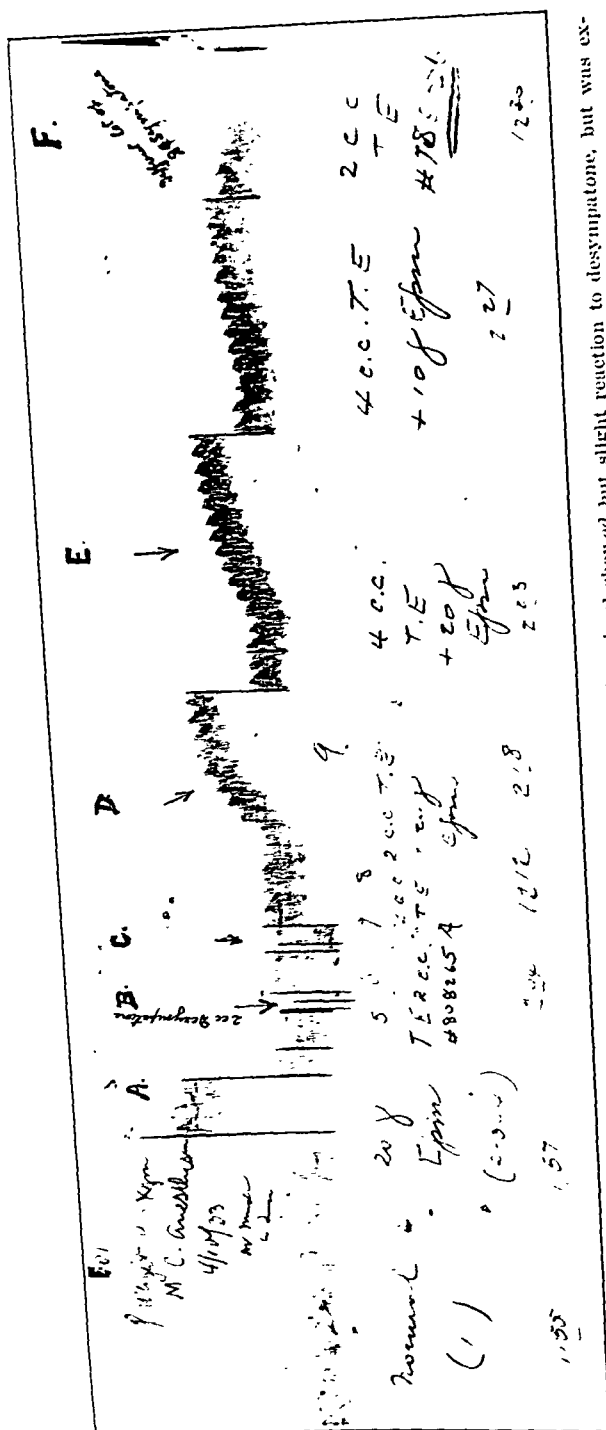


Fig. 1.—Showing effect of epinephrine and desympatone on Dog 301. This animal showed but slight reaction to desympatone, but was extremely sensitive to epinephrine.

only one ventricular response QRS, T; two normal cardiac cycles were again registered, followed once more by a ventricular standstill; this cardiac disturbance continued until it returned to its normal rhythm. The injection of

2 c.c. of tissue extract showed but a slight drop in blood pressure (Fig. 1, *B*); 4 c.c. of tissue extract also showed but a slight depressor effect (Fig. 1, *C*); 2 c.c. of tissue extract and 20 gamma of epinephrine brought about a marked rise in blood pressure (Fig. 1, *D*) and 4 c.c. of tissue extract still failed to

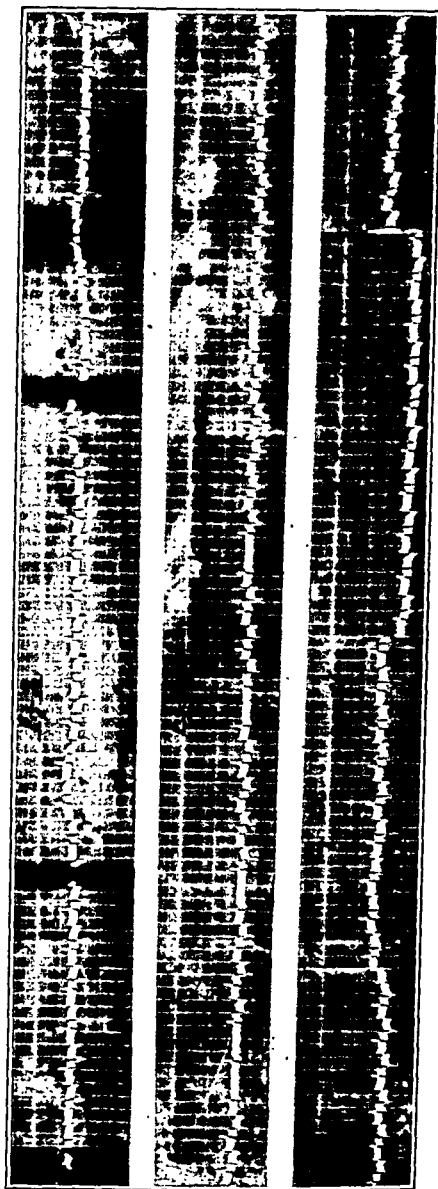


Fig. 2.—Normal electrocardiogram, Dog 301. *A*, Epinephrine produced numerous ventricular asystoles in this animal. *B* and *C*, Desympatone did not produce the characteristic effects upon the heart rate and T-waves in this animal. *D*, Desympatone administered with epinephrine did not influence the T-waves but prevented the ventricular asystoles seen in *A* when epinephrine alone was given. *E*, Desympatone from a different lot also failed to show the characteristic effects on this animal.

completely neutralize 10 gamma of epinephrine (Fig. 1, *E*). Taken after 2 and 4 c.c. of tissue extract respectively, the electrocardiograms (Fig. 2, *B* and *C*) did not show the characteristic effect upon the rate and T-waves. When the mixture of the two was administered (Fig. 2, *D*) tissue extract apparently prevented the blocking effect of epinephrine in this animal, but did not influence the T-wave. We have already shown in previous publications<sup>4, 5, 6</sup> that epineph-

rine and desympatone will affect the T-wave in opposite directions. In this animal the T-wave is not appreciably affected, since the T-wave in Lead 2 (Fig. 2), which we are continually registering, is very much like all the others seen in subsequent tracings.

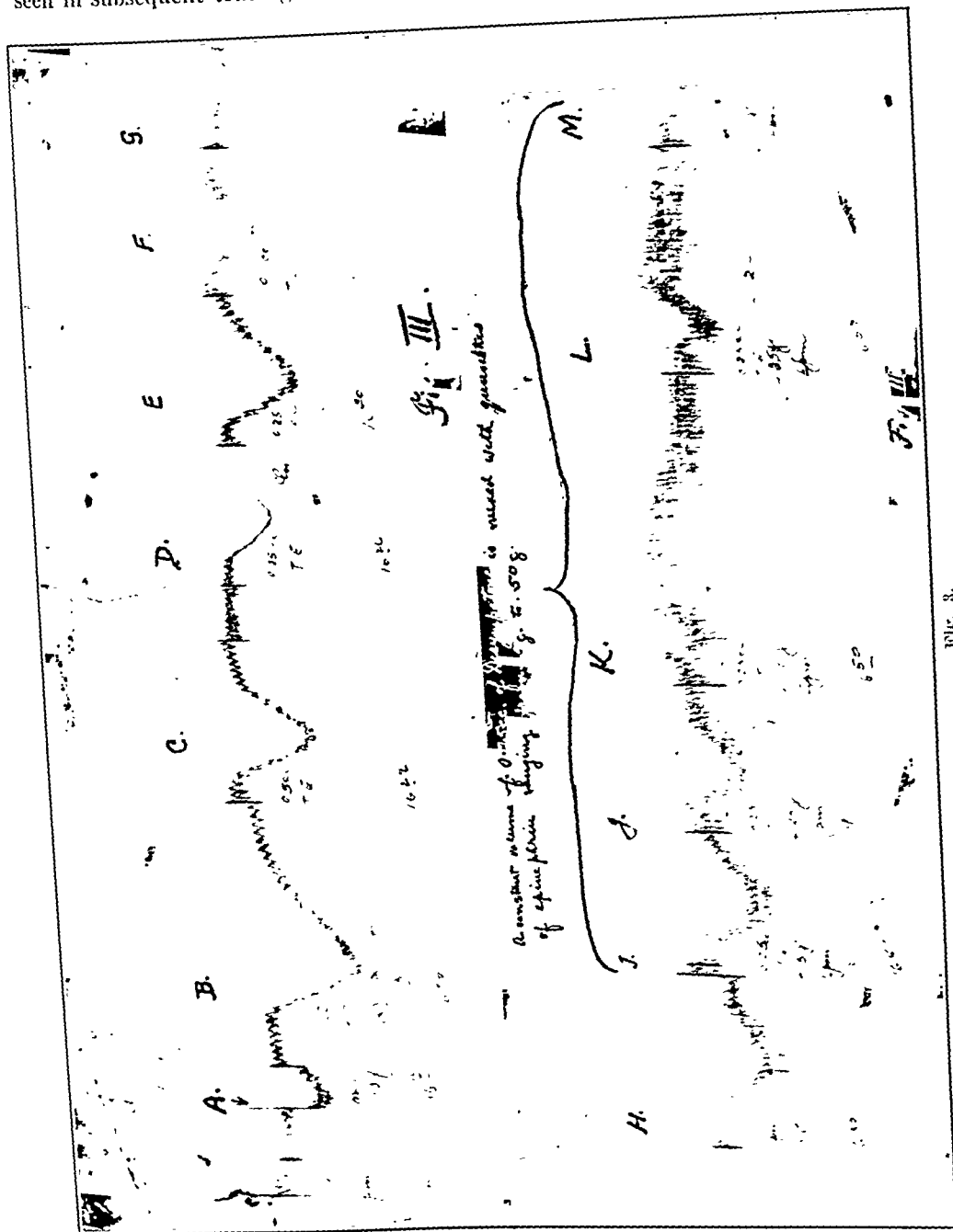


FIG. 2.

Thinking that possibly the tissue extract solution was weak, we used 2 c.c. from a different lot, but the effect upon the kymogram and electrocardiogram was about the same (Figs. 1, F and 2, E).

Figs. 3 and 4 show the blood pressure and electrocardiographic effects produced in Dog 302, following the administration of the same solutions. This animal proved to be very susceptible to desympatone and relatively resistant to epinephrine. Ten gamma of epinephrine caused a primary rise and secondary drop in the blood pressure (Fig. 3, A). The rate is uninfluenced. A slight

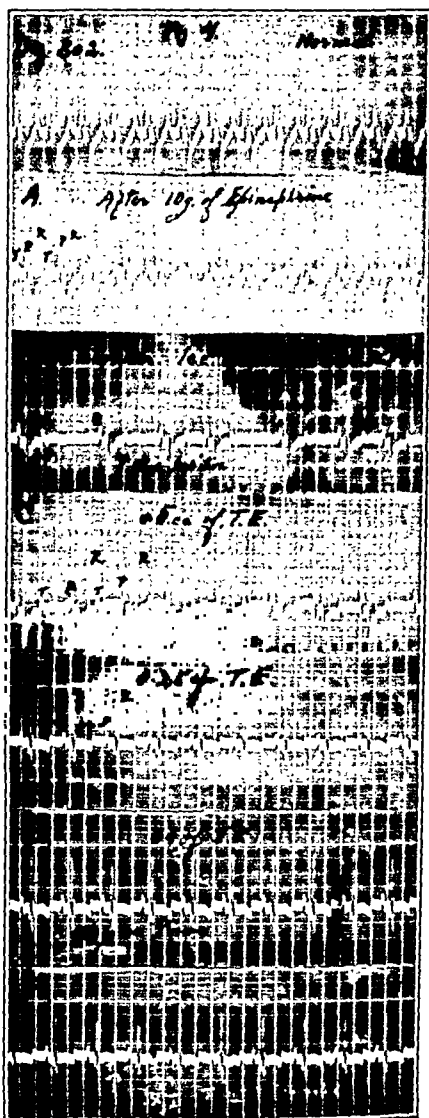


FIG. 4.

change developed in the character of the T-wave and in the R-T interval (Fig. 4, A). One cubic centimeter of tissue extract, employing the same lot used on the first animal, caused a marked depressor effect (Fig. 3, E). The electrocardiogram (Fig. 4, B) showed a definite sino-auricular bradycardia and sinus arrhythmia. The T-waves are markedly depressed as compared with the high T-wave seen on the normal tracing. The rate has been cut down from 144 to



84. Smaller amounts 0.5 c.c. (Figs. 3, *C* and 4, *C*) and 0.25 c.c. of tissue extract (Figs. 3, *D* and 4, *D*) showed proportionate drops and corresponding electrocardiographic changes. An injection of 0.25 c.c. of tissue extract (Figs. 3, *E* and 4, *D*) and 0.1 c.c. (Figs. 3, *F* and 4, *E*) again showed proportionate effects. As small a volume as 0.05 c.c. tissue extract (Fig. 3, *G*) showed a slight fall in blood pressure.

The method of standardization of tissue extract depends upon its neutralizing the effect of epinephrine upon the blood pressure of a dog. Fig. 3 (*I, J, K, L*, and *M*) shows the effects produced when a constant volume of 0.25 c.c. of tissue extract is mixed with quantities of epinephrine ranging from 5 gamma to 50 gamma. With 5 to 25 gamma of epinephrine the depressor action predominates in this dog. With 35 gamma (Fig. 3, *L*), the fall and rise are equivalent; and with 50 gamma of epinephrine, the pressor effect prevails (Fig. 3, *K*). Therefore, tested on this dog, 1 c.c. of tissue extract apparently corresponds to 120 gamma of epinephrine or 120 units, while if the previous dog was used as a test animal, 1 c.c. of the same solution would be equivalent only to 2.5 units, approximately 50 times less than in Dog 302. The very definite effect of repeated doses of desympatone on the T-wave should be noted. In the earlier records the T-wave was markedly upright (as shown by Fig. 4, *A x 1*) and later on it became definitely diphasic (Fig. 4, *D x 2*) and then inverted (Fig. 4, *F x 3*). The P-wave in the normal tracing is extremely high (Fig. 4, *A Y 1*), much smaller in Fig. 4, *B Y 2*, and proportionately increases and decreases, depending upon the amount of desympatone administered. In these two animals the differences in the results are extremely striking; in the first animal (301) 4 c.c. of tissue extract failed to neutralize 10 gamma of epinephrine, while in Dog 302, 0.25 c.c. of the same solution completely neutralized 35 gamma of epinephrine. In an assay on Dog 301 alone this solution would appear to contain less than 2.5 units per cubic centimeter, while assays on Dog 302 would indicate the presence of 140 units per c.c. These extreme variations are seldom seen. We avoid such discrepancies by using a number of anesthetized atropinized animals in standardizing tissue extract. Marked variations in susceptibility of the components of the autonomic system have frequently produced apparent contradictions in the results obtained during our studies. We have learned to determine the relative susceptibility of test animals<sup>7</sup> in connection with research work on products of this type, as well as in such apparently simple studies as the U.S.P.X. bioassay of epinephrine solutions.<sup>3</sup>

The difference in tonus of the different components of the autonomic system should be kept in mind in studying drugs having a specific effect upon this system. Many of the contradictory results observed by different investigators may be due to these marked differences in the autonomic balance of the animals employed.<sup>7</sup> A subsequent paper will present methods of grouping animals according to their autonomic states.

At present it appears important to test out every animal first as to its reaction to epinephrine. As soon as the effect of epinephrine has worn off, the

response to desympatone should be recorded. These hormones are very quickly eliminated,<sup>7</sup> apparently without influencing the animal's response to other drugs.

#### CONCLUSION

In studying the quantitative effects of drugs acting upon the autonomic system, the susceptibility of the test animals should be determined relatively to standard solutions of epinephrine and desympatone (the active constituent of tissue extract). Such a procedure will eliminate many of the apparent discrepancies observed on unstandardized animals.

We wish to acknowledge, with thanks, the splendid assistance of Arnold Quici and Mabel E. Luccareni for their assistance in compiling the necessary data, and to Sharp and Dohme for supplies of Tissue Extract 568.

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# INTRAVENOUS LETHAL DOSES OF AMYTAL IN THE DOG AND RABBIT AND A TABLE OF ANIMAL DOSAGES COMPILED FROM THE LITERATURE\*

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RECENT barbiturate studies indicate that closer attention must be paid to various details and factors than has hitherto generally been done in establishing accurate dosages. Also in some cases results cannot well be interpreted because authors give incomplete information about what would appear to be essential points. Finally, comparisons are difficult owing to variations in criteria and toxicologic terminology.

Therefore, we wish (1) to present a summary of recent studies upon factors which need consideration, together with our own findings in regard to some of them, (2) to discuss the possibility of clarifying the toxicology terminology, (3) to present a mortality study in which we have tried to follow the discussed principles, and (4) to give a comparative dosage table in which, by our headlines, the topics are indicated about which we believe information is desirable for proper evaluation of the proposed values.

## SUMMARY OF RECENT STUDIES

*The Drug Used, Its Degree of Purity, the Form in Which Administered, the  $P_H$ .*—"Crystalline sodium amytal has a lower M.L.D. than the prepared solution of amytal,"<sup>20a</sup> partly due to difficulties of avoiding slight decomposition and of changing all amytal into sodium amytal. Even pure sodium amytal crystals may contain not more than 0.1 per cent unchanged amytal.<sup>2</sup> Our dog experiments with intravenous, prepared sodium amytal do not give values that differ from those of Swanson and Shonle,<sup>32</sup> who used pure sodium amytal. Slight lowering of the intended  $P_H$  of 9.5 to 9.8 causes separation of traces of amytal with loss of hypnotic power.<sup>32, 35</sup>

Perfect stability of sodium amytal solutions of up to five months is claimed;<sup>18, 21, 24</sup> others note a tendency to deterioration on standing.<sup>32, 35</sup> Our pooled remnants after two months needed filtering and about 25 per cent increase in anesthetic dosage. Hence, for research work we use solutions on the day of preparation only.

A 10 per cent concentration is about 50 per cent more anesthetic in the white rat than a 2 per cent one, hypodermically administered.<sup>21</sup> Also, intravenous saline injections into rats revive those fatally poisoned with concen-

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trated solutions of sodium amytal.<sup>1</sup> On the other hand, rectal administration to dogs of 10 and 20 per cent solutions, respectively, give about similar results.<sup>32</sup>

As to *solvents*, ethyl glycol gives a more stable sodium amytal solution and dissolves amytal itself.<sup>21</sup> The pain factor necessitates rather dilute solutions for certain injections, and ethyl glycol must be studied further. A final acacia concentration of 10 per cent protects against circulatory effects (*Ibid.*).

In regard to *method of administration* and *rate of intravenous injection*, the rabbit shows an unusually large difference in lethal dosage between oral and other methods of administration.<sup>10</sup> Rabbits also survive operations better after oral than after subcutaneous administration.<sup>23</sup> And the lethal dose in this animal, and less so in the dog, declines very rapidly with increase in rapidity of intravenous administration.<sup>32</sup> Very rapid injection of but a few mls of even an animal's own blood may produce widespread disturbances, named "speed shock," with a possible early exitus.<sup>12</sup>

The total *number of animals*, and in mortality studies also the number in the fatal dose range, though not always available, would seem essential information for proper evaluation of any dosage. As to *sex*, adult female white rats are twice as susceptible to subcutaneous sodium amytal as are males, using deep anesthesia as a criterion,<sup>21</sup> but the two sexes are equally susceptible to nembutal.<sup>1</sup> Supposedly, the early *pregnancy* of 80 per cent of the amytal females is not changing the dose significantly. Rabbits do not show any sex difference when averaging many barbiturates after oral and intraperitoneal administrations;<sup>10</sup> the above nembutal report makes individual revision desirable. Very early *castration* makes the male need the male white rat dosage, whereas later castration reduces the dose needed;<sup>1</sup> however, the weekly production of anesthesia after early castration may have led to the development of some tolerance (*q. v.*). As to *age* and *size*, maturing male rats fall between adult males and females, and immature males and females give satisfactory results with female dosage.<sup>21</sup> In cats, 1.5 to 3.5 kg., the greater the weight, the slower the induction and the less the depth for the same dose per kilo, subcutaneously.<sup>20</sup> Our findings as to sex and size differences will be found under experimental data.

Regarding *external factors*, fluctuations in *room temperature* between 25° and 37° C. have not definitely influenced the M.L.D. of white rats in the fourteen series of experiments with intraperitoneal administration of 2 per cent solution of sodium amytal.<sup>32</sup> As to *excitement* and *fear* barbitate-treated rats, caged with a rabbit, though not attacked by it, remain awake considerably longer than the controls.<sup>13</sup> As much as 75 per cent dose differences with the same lot of white rats is "probably due to differences in strains of rats, seasonal changes, etc."<sup>26</sup> With groups of only 20 to 30 animals and no sex separation, any seasonal variation has yet to be established. Pending this, a direct comparison of amytal dosage of January with barbitate dosage of September<sup>31</sup> may be postponed. The possible influences of *diet*, *previous conditions of keeping animals*, and *barometric changes* are not reported upon. A *moist atmosphere* gives a more rapid recovery in monkeys treated with barbiturates than does a dry one.<sup>11</sup>

*Tolerance* of dog metabolism to amytal is not developed;<sup>4</sup> their example indicates intervals of about two weeks between administrations. Likewise, equally good anesthesia was obtained in fishes by repeating intraperitoneal injections up to five times, probably with ten-day intervals or more.<sup>15</sup> But repetition of injections within a few days of one another may necessitate twice the initial dose to produce anesthesia in the rat or death in the rabbit.<sup>9, 21</sup> Thus, probably, the immediately acquired tolerance is lost within a few weeks.

#### CLARIFICATION OF TOXICOLOGY TERMINOLOGY

(Comparisons of doses are further complicated because the toxicology terms do not mean the same to every investigator. Thus, the common term "minimum lethal dose" has variable meanings.<sup>33b</sup> It may mean a dose just sufficient to kill an occasional animal; e.g., Nielsen et al.<sup>22</sup> (1925) give increasing barbital doses to six cats, have the one on the highest dose die, and call that dose the M.L.D. Is it really justifiable to label any dose the M.L.D. from such an orientation experiment? Likewise Page and Coryllos<sup>24</sup> (1926) inject increasing amytal doses intravenously into nine dogs with fatal outcome to the one upon the highest dose, 95 mg. per kg.; they further state: "In one dose approximately 95-100 mg. per kilogram is fatal." No dose was tried between 75 and 95 mg. And the dosage is misprinted in a subsequent paper in which reference to the above gives the M.L.D. as 110 mg. per kg.<sup>31</sup> (Their Table VI.)

✓To Maloney, Fitch and Tatum<sup>19</sup> (1931) the M.L.D. causes death in 50 per cent of the animals, whereas Knoefel, Herwick and Loevenhart<sup>16</sup> (1930) define it as the smallest dose which kills over 50 per cent of the cases.

According to Swanson<sup>29</sup> (1932, a), "the M.L.D. is determined by requiring not less than 60 per cent (of the rats) to die on the same dose." Shonle, Keltch and Swanson<sup>26</sup> (1930) write that the M.L.D. is that which causes death of all or a majority of the animals on that dose. In the amytal series of Coutière<sup>3</sup> (1932) it seems to be the dose just large enough to kill every animal on that dose.

Similar divergence holds for the term "average fatal dose."<sup>7, 17, 32</sup>

To eliminate some less useful new barbiturates one may divide 350 rats among 16 barbiturates to get a rough idea of the effective and fatal dosage,<sup>22</sup> and even in a few cases use a rat a second time with at least a two-week interval.<sup>25</sup> But though there be a distinct advantage in using the values from one comprehensive piece of work for comparative studies, it does not seem quite justifiable to employ the values of the just-mentioned investigators for detailed comparison among the more important barbiturates, including the effects of varying percentages of the so-called M.L.D.<sup>30</sup> The number of animals is too small, the concentration is variable and nothing is said about a constant sex ratio. Even the revision of this comparative dosage,<sup>29b</sup> though using more animals, fails to consider the sex question and to keep a constant concentration.

✓In view of these difficulties of nomenclature we agree with Trevan<sup>33</sup> (1927) that it would be preferable to abolish the term "Minimum Lethal Dose" and to substitute a term that would always mean the same. He proposes the term

*median lethal dose* to designate a dose which will kill 50 per cent of all animals in question in a large group picked at random, and suggests the abbreviation L D 50 for it. L D 100 would be an unequivocal abbreviation for the certainly fatal dose. Proofs are given that the L D 50 is "in the neighborhood of doses with the minimum relative error of determination." Regarding the number of animals required in such determinations, mathematical evidence is furnished to show that about 30 is near the optimum, that significant increase in accuracy requires a great increase in number, and that "diminution of the group below 30 leads to a very rapidly increasing error." Or, as Trevan puts it: "The ratio of the cost of the test to the accuracy obtained reaches a minimum in the neighborhood of this group number." However, with larger numbers of animals, one may determine several points along the straight part of the mortality curve, usually between about L D 20 and L D 80, by splitting up this number into smaller groups, and get results "which will have only a slightly greater error than that which is obtained by injecting the whole group with the average lethal dose." The total number required for the determination of these points depends partly upon the slope of the characteristic and partly upon the accuracy desired. Trevan gives examples of this and adds emphatically that all "discrepant" results must be included. He<sup>33</sup> further writes (1932, a): "The ideal way is to get a set of groups symmetrically distributed around 50 per cent mortality with doses separated by intervals such that the difference of the logarithms is constant, the doses themselves, therefore, rising by equal proportions and not equal arithmetical differences. The L D 50 can be arrived at by drawing on squared paper the best straight line through the points obtained and determining where it cuts the line for 50 per cent mortality."

As to establishing the minimum effective dose, except when actual surgical operation is the aim, criteria vary so greatly both for the same and for other species that comparisons of results of different authors become quite difficult. Could not the Council or some other authoritative body suggest one or two criteria for each species of animals by which first to establish the M.E.D. in each case of new hypnotics, the stream of which as yet seems far from being stemmed?

#### EXPERIMENTAL DATA

The solution of sodium amytal was prepared in the usual manner, adding 8.85 mil. of N/2 pure sodium hydroxide solution to each gram of amytal with proper stirring, avoiding excessive heating, and with subsequent dilution by freshly glass-distilled, sterile water so as to make the concentration 10 per cent of sodium amytal. The solutions were always filtered, kept between 24° and 28° C. during use, and employed within six hours. On three occasions the  $P_H$  was checked and found to be within the range of 9.5 to 9.8.

Our original plan called for completion of all experiments during the warm months of October and November, but an outbreak of plague interrupted our animal supply and made it necessary to continue into December and January; however, the estimated L D 50 for the 109 dogs of the warmer months was only one milligram higher than that of the 71 of the colder ones, and in the case

of the rabbits a similar difference was found; hence, no important source of error was introduced by the change of season. So as to have more uniform temperature conditions during the actual experiments, the room was warmed and the temperature kept between 21° and 27° C. and the animals were kept well covered during the amytal action.

The short-haired varieties of rabbits were bought in the open market, and kept for at least ten days on a constant diet of wheat, carrots, fresh greens, and water. A total of 120 rabbits was used, 60 males and 60 nonpregnant females. Food, but not water was removed twenty hours before giving amytal.

The 180 dogs were delivered from the city pound, all breeds and mixtures, 90 males and 90 nonpregnant females, and kept on a mixed diet; as much

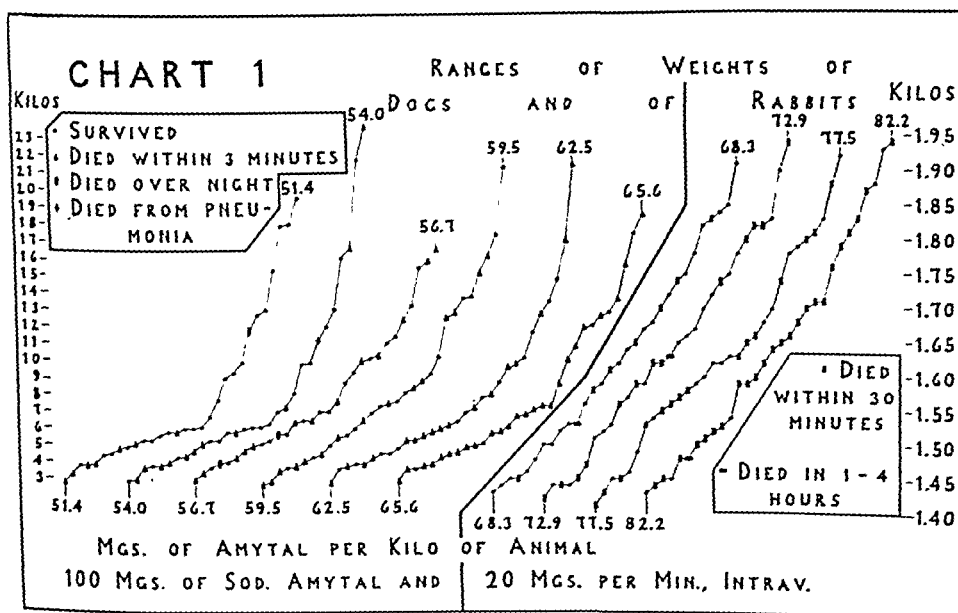


Chart 1.—Showing the weight ranges and uniformity of distribution of weights within each range; the size, dose, fate, method of administration and rate of injection for each and every animal (100 mg. per minute for dogs, 20 for rabbits); the increasing mortality with increasing doses, the very few delayed deaths and the higher mortality in the small dogs in the first four series.

bread and boiled meat as they would eat was given twenty-four hours before administering amytal; water was allowed all the time.

The determinations were completed in ten days upon the rabbits and in eleven days upon the dogs. On each day we tried to distribute the animals evenly among the doses.

The rabbit variation in weight was limited to the narrow range of between 1.40 and 1.95 kg. In the dogs, using only full-grown, apparently healthy animals, the weight range is that of the animals brought to us, with extremes of 2.7 and 23.4 kg. Chart 1 shows that the four series of 30 rabbits each, one for each dose, are very similar in their size distribution. In the six dog series the size distribution is sufficiently similar for biologic experiments.

Because rabbits are especially susceptible to very fast injections, we injected only 20 mg. of sodium amytal per minute into the marginal ear vein,

giving a minimum time of five minutes per rabbit. In case of the dogs the rate was 100 mg. per minute into the external saphenous vein. We are purposely giving our rate in terms of sodium amytal, so that later direct comparisons with pure sodium amytal mortalities may be more readily carried out.

In the rabbit the increase in dosage is 5 mg. of sodium amytal per kilogram for each new series. In the dogs, the dose increases in steps of 5 per cent (Logarithm 0.02119), so as to follow the latest suggestion by Trevan; this percentage increase seems to be very suitable for this type of medication. In either case the distribution around 50 per cent mortality was very satisfactory.

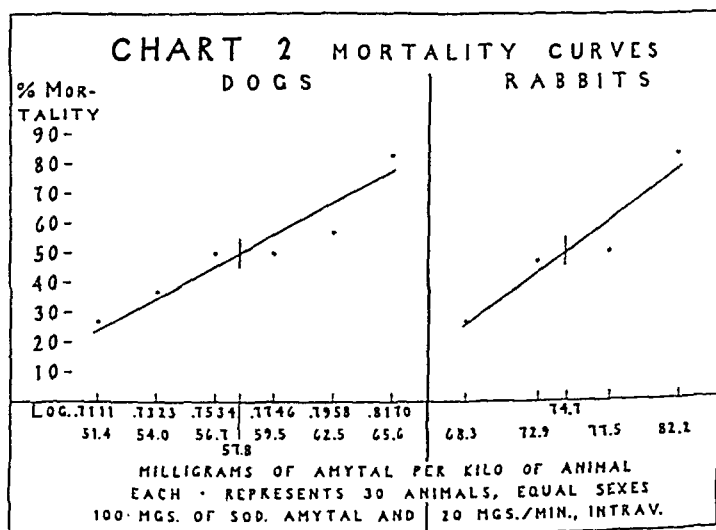


Chart 2.—Showing the percentage mortality of thirty animals on each of the indicated doses, the place where the best straight line fitting the points cuts the 50 per cent mortality line (L D 50) and the suitability of 5 per cent dose increases for intravenous amytal administration.

Our main results are shown in Charts 1 and 2 and in Tables I and II.

In view of the rather few mortality percentage points we have thought it best to draw the straight line that would best fit these, and thus to determine the L D 50 by seeing where this line would cut the 50 per cent mortality line. Because there seems to be a flattening in the middle values, one may try to use other types of curves; however, this will not appreciably alter the L D 50 value.

TABLE I

PERCENTAGE MORTALITIES IN RABBITS, 20 MG. OF SODIUM AMYTAL PER MINUTE

| AMYTAL<br>MG./KG.                                  | 15<br>MALES | 15<br>FEMALES | TOTAL<br>30 |
|----------------------------------------------------|-------------|---------------|-------------|
| 68.3                                               | 27          | 27            | 27          |
| 72.9                                               | 73          | 20            | 47          |
| 77.5                                               | 33          | 67            | 50          |
| 82.2                                               | 87          | 80            | 83          |
| All doses combined: 60 males 55 per cent mortality |             |               |             |
| 60 females 48 per cent mortality                   |             |               |             |
| 120 (total) 52 per cent mortality                  |             |               |             |



TABLE II

PERCENTAGE MORTALITIES IN DOGS, 100 MG. OF SODIUM AMYTAL PER MINUTE

| AMYTAL<br>MG./KG.                                  | 15<br>MALES | 15<br>FEMALES | TOTAL<br>30 |
|----------------------------------------------------|-------------|---------------|-------------|
| 51.4                                               | 27          | 27            | 27          |
| 54.0                                               | 47          | 27            | 37          |
| 56.7                                               | 53          | 47            | 50          |
| 59.5                                               | 40          | 60            | 50          |
| 62.5                                               | 67          | 47            | 57          |
| 65.6                                               | 87          | 80            | 83          |
| All doses combined: 90 males 53 per cent mortality |             |               |             |
| 90 females 48 per cent mortality                   |             |               |             |
| 180 (total) 51 per cent mortality                  |             |               |             |

To the nearest milligram of amytal itself, the L D 50 for the rabbits with our rate of 20 mg. of sodium amytal per minute, intravenously, is 75 mg. per kg., and for the dogs, using 100 mg. per minute, 58 mg. per kg. Comparisons with the rabbit values of Knoefel<sup>16</sup> et al. (1930; 68 mg.) and of Coutière<sup>3</sup> (1932; 65 mg.) are impracticable because they did not use a fixed rate of injection, and employed much fewer animals (see Table III); Swanson and Shonle's<sup>32</sup> (1931) pure sodium amytal value of 86 mg. of amytal is based upon one breed of rabbits, a faster rate and lower concentration, and Barron's<sup>1</sup> (1933) recent report makes it highly necessary to consider the concentration even with intravenous medication of amytal. In case of the dog our value checks very well with that of Swanson and Shonle<sup>32</sup> (1931) for pure sodium amytal; they report an M.L.D. (over 60 per cent fatality) of 63 mg. of amytal per kg.; 63 mg. from our curve gives an L D 67; concentration and injection rate were the same in both cases. A tentative value for the L D 25 in the rabbit would be 68, and for the dog 52 mg. of amytal per kg. Similarly, a tentative value for the L D 75 in the rabbit is 81, and in the dog 65 mg. of amytal per kg. with our injection rates. Thus, in either case, an increase of 13 mg. of amytal per kg. increases the fatalities from 25 per cent to 75 per cent.

In case of the rabbits, 59 out of 62 (or 95 per cent) died within thirty minutes, and 3 (or 5 per cent) died in from one to four hours after completion of injection (Chart 1). In case of the 91 dog fatalities, 88 (97 per cent) succumbed within three minutes of finishing the injection, whereas the remaining 3 (3 per cent) died during the night. In most cases the acute death in the dogs was immediately preceded by a short period of marked hyperpnea. Both acute and subacute deaths have been included in the mortality percentages as being directly attributable to amytal. One dog had evidently recovered from amytal after twenty-four hours; however, it developed pneumonia and died on the third day; this we have classed as an amytal survival. Similarly, another one of our survivals had pneumonia, but made a perfect recovery. The rabbits were kept for at least ten days after the experiment; none died during this time nor did any show signs of being ill. The dogs were killed at varying intervals of time (from one to eleven days) after recovery, to be used in a study of any possible pathologic changes (see later discussion).

In regard to any possible sex difference in the rabbit, Table I shows the extreme variations one may expect when using only 15 animals in one group

TABLE  
AMYTAL DOSE

| ANIMALS AND INVESTIGATORS | NUMBER OF<br>ANIMALS, FATAL<br>RANGE IN ( ) | SIZES IN GM. OR KG.<br>SEXES                                       | SUBSTANCE: PRE-<br>PARED, OR PURE<br>SOD. AMYTAL | PERCENTAGE<br>CONCENTRA-<br>TION |
|---------------------------|---------------------------------------------|--------------------------------------------------------------------|--------------------------------------------------|----------------------------------|
| <i>Fishes: (b)</i>        |                                             |                                                                    |                                                  |                                  |
| Keys and Wells            | 35 (15)                                     | Wide range                                                         | Prepared                                         | 1                                |
| <i>White Mice:</i>        |                                             |                                                                    |                                                  |                                  |
| Dox and Hjort             |                                             |                                                                    | Prepared                                         | 1                                |
| <i>White Rats:</i>        |                                             |                                                                    |                                                  |                                  |
| Nielsen, et al.           | About 23                                    |                                                                    | Prepared                                         | 1 and less                       |
| Swanson and Page          | (39) 26                                     | 123-285                                                            | Prepared                                         | 10                               |
| Nicholas and Barron       | 250 pregn. and<br>45 nonpregn.<br>About 200 | 150-250<br>Females<br>Over 200<br>Males<br>Males<br>Males<br>Males | Pure<br>Pure<br>Pure<br>Pure<br>Pure<br>Pure     | 10<br>5<br>2.5<br>2.0<br>2<br>5  |
| Swanson                   | 55 (55)                                     |                                                                    | Pure                                             | 2                                |
| Hoskins, et al.           | 33                                          |                                                                    | Prepared                                         | 5                                |
| Drapkin, et al.           |                                             | 200                                                                | Prepared                                         | (d)                              |
| Swanson and Shonle        | 936 (169)                                   | About 60% Males                                                    | Pure                                             | 2                                |
| Shonle, et al.            | 18                                          | 100                                                                | Prepared                                         | 2                                |
| Fitch and Tatum           | Over 40                                     |                                                                    | Prepared                                         | 5                                |
| Hoskins, et al.           | 24                                          | Young                                                              | Prepared                                         |                                  |
| <i>Rabbits:</i>           |                                             |                                                                    |                                                  |                                  |
| Fitch and Tatum           | Over 40                                     |                                                                    | Prepared                                         |                                  |
| Page                      |                                             |                                                                    | Prepared                                         | 5                                |
| Swanson and Page          | 94 (19)                                     | 1.1-2.3                                                            | Prepared                                         | 10                               |
| Page                      | 8                                           | 1.3-2.7                                                            | Prepared                                         | 10                               |
| Shonle and Moment         |                                             |                                                                    | Prepared                                         | 10                               |
| Dox and Hjort             | Over 40                                     |                                                                    | Prepared                                         | 10                               |
| Fitch and Tatum           | 16 ( 16)                                    |                                                                    | Prepared                                         | 5                                |
| Knoefel, et al.           | 23 ( 23)                                    |                                                                    | Pure                                             | 5                                |
| Swanson and Shonle        | 15 ( 15)                                    |                                                                    | Pure                                             | 5                                |
|                           | 8 ( 8)                                      |                                                                    | Pure                                             | 5                                |
|                           | 10 ( 10)                                    |                                                                    | Pure                                             | 5                                |
|                           | 8 ( 8)                                      |                                                                    | Pure                                             | 1.3-2                            |
| Coutière                  | 29 ( 18)                                    | 1.8-2.5                                                            | Prepared                                         | 10                               |
| Holck and Kan'an          | 120 (120)                                   | 1.4-2.0<br>Equal sexes                                             | Prepared                                         |                                  |
| <i>Cats:</i>              |                                             |                                                                    |                                                  |                                  |
| Swanson and Page          | 20 ( 20)                                    | 1.9-2.5                                                            | Solid Am.                                        | Dilute                           |
| Eddy                      | 10 ( 8)                                     | Adults                                                             | Amytal suspen-<br>sion with acacia               |                                  |
| Mulinos                   | 21                                          | 1.3-3.5<br>43% Males                                               | Prepared                                         | 10                               |
|                           | 33                                          |                                                                    | Prepared                                         | 10                               |
| Koppanyi and Lieberman    | 15 ( 11)                                    |                                                                    | Pure                                             | 10                               |
| <i>Dogs:</i>              |                                             |                                                                    |                                                  |                                  |
| Swanson and Shonle        | 76 ( 31)                                    | Adults                                                             | Pure                                             | Solid<br>(capsules)              |
| Nielsen, et al.           |                                             |                                                                    | Amytal                                           | Solid<br>(capsules)              |
| Page                      |                                             |                                                                    | Prepared                                         | 10                               |
| Swanson and Shonle        | 80 ( 63)                                    | Adults                                                             | Pure                                             | Less than 10<br>and 20           |
| Page                      |                                             |                                                                    | Prepared                                         | 10                               |
| Edwards and Page          |                                             | Variable                                                           | Prepared                                         | 10                               |
|                           |                                             |                                                                    | Prepared                                         | 10                               |

### AGE TABLE

[illegible]

TABLE III

| ANIMALS AND INVESTIGATORS | NUMBER OF<br>ANIMALS, FATAL<br>RANGE IN ( ) | SIZES IN GM. OR KG.<br>SEXES | SUBSTANCE: PRE-<br>PARED, OR PURE<br>SOD. AMYTAL | PERCENTAGE<br>CONCENTRA-<br>TION |
|---------------------------|---------------------------------------------|------------------------------|--------------------------------------------------|----------------------------------|
| Holck and Kanán           | 39                                          | 3-18 Adult<br>Females        | Pure (e)                                         | 10                               |
|                           | 26                                          | 3-20 Adult<br>Males          | Pure                                             | 10                               |
| Swanson and Shonle        | 73 (29)                                     |                              | Pure                                             | 10                               |
|                           | 27                                          |                              | Pure                                             | 10                               |
|                           | 25                                          |                              | Pure                                             | 10                               |
|                           | 15                                          |                              | Pure                                             | 10                               |
|                           | 15                                          |                              | Pure                                             | 10                               |
|                           | 15                                          |                              | Pure                                             | 10                               |
| Holck and Kanán           | 180 (180)                                   | 3-23 Adults<br>Equal sexes   | Prepared                                         | 10                               |
| <i>Lower Primates</i>     |                                             |                              |                                                  |                                  |
| Fulton and Keller         | Several                                     |                              | Prepared                                         | 10                               |
| Weidman                   | 2                                           |                              | Prepared                                         | 10                               |

(a) All doses are in amytal as such. Pure sodium amytal has been multiplied by the factor given by Swanson, 0.002, to give amytal. All rates in intravenous injections are in milligrams of sodium amytal.

(b) The species were: *Girella Nigricans*<sup>11</sup>; *Clinocottus Analis*<sup>11</sup>; *Apomatis Cyanellus* and *Caresius Auratus*, a few of each.

(15 males or 15 females). The reversal of the high mortality for the two sexes in the two middle groups with good similarity in the lowest and highest dosage, indicates that there was no clear-cut sex difference. Similarly, in the case of the dogs, one cannot draw any definite conclusion from Table II. In view of the fact that in both cases the combined survivals on all doses somewhat favor the females (16 per cent more females survive in case of the rabbits and 11 per cent in case of the dogs), and because we have had to increase the sodium amytal dose by 5 mg. above the initial 60 per kg. more frequently in the females than in the males to produce surgical anesthesia (see Table III), it may be worth while to study this possible sex difference upon a large number of animals.

Although the fatalities do not with certainty show any sex difference, Tables IV and V show that on the whole the female rabbits do not have their body temperatures nor their respiratory rates quite as much depressed as do the males, and that they recover faster than the males. Although it is not very satisfactory to speak of a standard deviation for a few animals, our values also indicate that some males recover as fast as the females, but most do not. We have only included the rabbits of the warmer months, because with temperatures of about 21° C. not only are the initial body temperatures and especially respiratory rates less, but the temperature falls are more marked and complicated by shivering. The standard deviations also bring out the enormous variations between recoveries in rabbits who have been given the same dose and are lying side by side. Furthermore, actually the lowest respiratory rate came in our readings at fifteen, thirty, or forty-five minutes, so respiration rates begin to recover at a time when body temperatures are still falling (low point usually at second hour reading). Of course, most of the early workers with amytal reported depressed respiration rates and body temperatures, but as far as we know none reported upon any sex differences.

-CONT'D

| METHOD OF ADMINISTRATION AND MG. OF SOD. AMYTAL/MIN. | MIN. EFFECTIVE DOSE AS AMYTAL MG./KG. (a) | CRITERION AB = ABOLISHED R = REFLEX | LETHAL DOSES AS AMYTAL MG./KG. | CRITERION       | REMARKS ON (1) STARVATION IN HOURS (2) DIET (3) ROOM TEMP. IN C° (4) SEASON (5) MISCELLANEOUS |
|------------------------------------------------------|-------------------------------------------|-------------------------------------|--------------------------------|-----------------|-----------------------------------------------------------------------------------------------|
| Intrap.                                              | 54                                        | Anesthesia in 72%                   |                                |                 | (1) Mixed                                                                                     |
| Intrap.                                              | 50                                        | Anesthesia in 90%                   |                                |                 | (2) 24                                                                                        |
| Intrap.                                              | 54                                        | Anesthesia in 92%                   |                                |                 | (3) All year                                                                                  |
| Intrap.                                              | 50                                        | Anesthesia in 90%                   |                                |                 | (4) May                                                                                       |
| Intrap. 100                                          | 36-45                                     | Anesthesia                          | 63                             | 60% or more die | (4) August                                                                                    |
| Intrap. 200                                          |                                           |                                     | 68                             | 60% or more die |                                                                                               |
| Intrap. 500                                          |                                           |                                     | 68                             | 60% or more die |                                                                                               |
| Intrap. 750                                          |                                           |                                     | 63                             | 60% or more die |                                                                                               |
| Intrap. 1500                                         |                                           |                                     | 50                             | 60% or more die |                                                                                               |
| Intrap. 2000                                         |                                           |                                     | 54                             | 60% or more die | (1) 24; (2) Mixed                                                                             |
| Intrap. 100                                          |                                           |                                     | 58                             | L D 50          | (3) 21-27                                                                                     |
|                                                      |                                           |                                     |                                |                 | (4) Oct.-Jan.                                                                                 |

|         |       |            |
|---------|-------|------------|
| Intrap. | 50-70 | Anesthesia |
| Intrap. | 55    | Anesthesia |

- (c) Anesthesia is used to designate complete, deep or surgical anesthesia or narcosis. Sedative and light sleep doses have been omitted.
- (d) It seems probable but not certain that 10 per cent was used whenever no concentration is given for injected sodium amytal. This does not hold for oral administration in which probably extra water was given to the animal to rinse the stomach tube.
- (e) Courtesy of the Eli Lilly Company.

As to the possible effect of variability in size, a careful comparison between the mortality percentage of the heavier 50 per cent (from 1.62 to 1.94 kg.) and the lighter 50 per cent (from 1.41 to 1.62 kg.), and, similarly, between the 10 heaviest and the 10 lightest within each of the four rabbit series, shows clearly that within the given limits of weights the size was not important in regard to fatality upon any given dose. This may also be seen by looking at the

TABLE IV  
CHANGES IN RABBITS' BODY TEMPERATURES UNDER INTRAVENOUS FATAL RANGE DOSES OF SODIUM AMYTAL  
ROOM TEMPERATURES 24.5 TO 27.0° C.

| FEMALES | AVERAGE OF 7<br>(75 MG./KG.) |                  | AVERAGE OF 9<br>(80 MG./KG.) |                  | AVERAGE OF 4<br>(85 MG./KG.) |                  |
|---------|------------------------------|------------------|------------------------------|------------------|------------------------------|------------------|
|         | DEGREES<br>C.                | ST.<br>DEVIATION | DEGREES<br>C.                | ST.<br>DEVIATION | DEGREES<br>C.                | ST.<br>DEVIATION |
| Normal  | 39.3                         | 0.3              | 39.2                         | 0.4              | 39.6                         | 0.1              |
| 1 hour  | 37.3                         | 0.6              | 37.1                         | 0.7              | 37.3                         | 0.3              |
| 2 hours | 37.2                         | 0.6              | 37.0                         | 0.6              | 37.3                         | 0.4              |
| 3 hours | 38.2                         | 0.8              | 37.7                         | 0.8              | 38.2                         | 0.5              |
| 4 hours | 38.0                         | 0.6              | 38.2                         | 0.7              | 39.2                         | 0.3              |
| 5 hours | 39.2                         | 0.5              | 38.8                         | 0.5              | 39.3                         | 0.2              |
| 6 hours | 39.4                         | 0.5              | 39.2                         | 0.3              | 39.3                         | 0.2              |

| MALES   | AVERAGE OF 8<br>(75 MG./KG.) |                  | AVERAGE OF 3<br>(80 MG./KG.) |                  | AVERAGE OF 10<br>(85 MG./KG.) |                  |
|---------|------------------------------|------------------|------------------------------|------------------|-------------------------------|------------------|
|         | DEGREES<br>C.                | ST.<br>DEVIATION | DEGREES<br>C.                | ST.<br>DEVIATION | DEGREES<br>C.                 | ST.<br>DEVIATION |
| Normal  | 39.2                         | 0.2              | 39.5                         | 0.1              | 39.0                          | 0.3              |
| 1 hour  | 36.9                         | 0.4              | 36.6                         | 0.4              | 36.5                          | 0.8              |
| 2 hours | 36.6                         | 0.6              | 36.0                         | 0.2              | 36.5                          | 0.8              |
| 3 hours | 36.7                         | 0.8              | 35.9                         | 0.1              | 36.9                          | 1.1              |
| 4 hours | 37.0                         | 1.0              | 36.2                         | 0.4              | 37.3                          | 1.4              |
| 5 hours | 37.8                         | 1.2              | 37.2                         | 1.4              | 37.9                          | 1.4              |
| 6 hours | 38.5                         | 0.9              | 37.6                         | 1.1              | 38.2                          | 1.2              |

TABLE V

CHANGES IN RABBITS' RESPIRATION RATES UNDER INTRAVENOUS FATAL RANGE DOSES OF SODIUM AMYTAL

ROOM TEMPERATURES 24.5 TO 27.0° C.

| FEMALES | AVERAGE OF 7<br>(75 MG./KG.) |                  | AVERAGE OF 9<br>(80 MG./KG.) |                  | AVERAGE OF 4<br>(85 MG./KG.) |                  |
|---------|------------------------------|------------------|------------------------------|------------------|------------------------------|------------------|
|         | RESP.<br>RATES               | ST.<br>DEVIATION | RESP.<br>RATES               | ST.<br>DEVIATION | RESP.<br>RATES               | ST.<br>DEVIATION |
| Normal  | 228                          | 35               | 236                          | 12               | 224                          | 7                |
| 1 hour  | 37                           | 15               | 23                           | 9                | 23                           | 1                |
| 2 hours | 51                           | 24               | 26                           | 8                | 38                           | 20               |
| 3 hours | 74                           | 34               | 43                           | 38               | 108                          | 43               |
| 4 hours | 134                          | 65               | 78                           | 55               | 194                          | 33               |
| 5 hours | 171                          | 64               | 133                          | 56               | 220                          | 25               |
| 6 hours | 219                          | 35               | 193                          | 40               | 259                          | 22               |

| MALES   | AVERAGE OF 8<br>(75 MG./KG.) |                  | AVERAGE OF 3<br>(80 MG./KG.) |                  | AVERAGE OF 10<br>(85 MG./KG.) |                  |
|---------|------------------------------|------------------|------------------------------|------------------|-------------------------------|------------------|
|         | RESP.<br>RATES               | ST.<br>DEVIATION | RESP.<br>RATES               | ST.<br>DEVIATION | RESP.<br>RATES                | ST.<br>DEVIATION |
| Normal  | 221                          | 21               | 235                          | 16               | 237                           | 26               |
| 1 hour  | 30                           | 10               | 18                           | 7                | 22                            | 5                |
| 2 hours | 32                           | 7                | 24                           | 6                | 32                            | 15               |
| 3 hours | 35                           | 9                | 30                           | 3                | 46                            | 26               |
| 4 hours | 49                           | 15               | 35                           | 11               | 68                            | 49               |
| 5 hours | 84                           | 50               | 71                           | 49               | 87                            | 57               |
| 6 hours | 115                          | 61               | 93                           | 57               | 115                           | 67               |

distribution of fatalities along the total range of weights for each of the four rabbit doses (Chart 1). On the other hand, in case of the dogs there is a higher mortality in the weight range of from 2.7 to 5.4 kg. (66 dogs) in the lower four dosages as compared with the range of either from 5.5 to 8.9 kg. (55 dogs), or 9.0 to 23.5 kg. (59 dogs). The curve for the group of smallest dogs starts at a much higher level and is much less steep than either of the other two (actual mortality percentages for each of the six doses is 50, 58, 60, 60, 55, and 82, respectively, for this group). The main reason for this difference would seem to be the fact that in the smallest dogs the total dose would have been given in a very much shorter time than in case of a large one; thus, the very fastest total time for any one dog was one and six-tenths, and the very slowest was fourteen and six-tenths minutes, whereas in case of the rabbits the values are five and three-tenths and eight and eight-tenths minutes, respectively. It is also possible that the very small dogs may be more of the lap or house-pet type, and therefore be slightly less vigorous. The difference is not due to any very unequal sex distribution within this group of small dogs (19 males and 25 females in the four lower groups).

To get an approximate idea of what the L D 50 would be in the dog in the absence of extreme body weight variations, we have examined mortality curves for the middle two-thirds of each series (total of 120 dogs; approximate weight range of from 4 to 13.6 kg.), for the exact weight range of from 4.5 to 13.5 kg. in each series (117 dogs), and for the exact range of from 5.0 to 12.5 kg. within each series (96 dogs with not less than 15 in any of the six

groups). The L D 50 determined from each of these curves is 59 mg. of amytal per kg. of dog, or only 1 mg. higher than that for the total weight range.

Ravdin, Drabkin, and Bothe<sup>25</sup> (1931) report that repeated injections of good doses of sodium amytal into the white rat had no detrimental effect upon the heart, liver, lung, and kidney as judged by microscopic examination of these organs. In order to see whether or not lethal dose ranges of intravenously given sodium amytal injured the kidney or liver, sections were made from a large number of our dogs and stained with hemotoxylin and eosin. The slides from those that died acutely and those that were killed from one to eleven days after having been given the drug were then compared with the tissues of 10 apparently normal dogs which were killed by shooting. Table V shows that our street dogs, though apparently healthy, do show a good deal of pathologic changes.

TABLE VI

SUMMARY OF PATHOLOGIC CHANGES IN THE KIDNEY AND LIVER IN NORMAL AND IN AMYTAL-TREATED DOGS

|                                           | NUMBER<br>OF | BOTH ORGANS<br>NORMAL | SLIGHT CHANGES<br>IN ONE OR BOTH | MORE SEVERE<br>CHANGES IN ONE OR<br>BOTH ORGANS |
|-------------------------------------------|--------------|-----------------------|----------------------------------|-------------------------------------------------|
| "Normal" dogs                             | 10           | 50%                   | 30%                              | 20%                                             |
| Acute amytal deaths                       | 51           | 33%                   | 50%                              | 17%                                             |
| Killed one to eleven<br>days after amytal | 55           | 33%                   | 50%                              | 17%                                             |

Dr. Homma reports: "The changes found in the liver were parenchymatous degeneration and fat infiltration and periportal round cell infiltration; in the kidneys, parenchymatous degeneration and interstitial round cell infiltration.

"All these changes, except the interstitial round cell infiltration in the kidney, which was found three times only among the dogs treated with amytal, were also found among the supposedly normal dogs. Also, no relationship between the changes and the dose of amytal given or the time after the administration could be established. So it must be concluded that the pathologic findings in the liver and kidney were not due to amytal."

#### SUMMARY

1. The introduction discusses the relative merits of the various factors which may modify amytal dosage and the desirability of clarifying the toxicology terminology by the adoption of the median lethal dose (L D 50) as a common criterion of fatality, and gives suggestions as to the manner of conducting mortality studies.

2. Ten per cent freshly prepared sodium amytal solution was injected intravenously at a rate of 20 mg. per minute of sodium amytal per kg. into each of 120 rabbits, equal sexes, from 1.4 to 2.0 kg., divided into four groups of thirty each, and using differences of 5 mg. of sodium amytal between the groups. From the best straight line that fits the four mortality percentage points, the L D 50 comes out to be 75 mg. of amytal itself per kg. of rabbit.

The value is not readily comparable with those of other reports, for others used lower concentrations and faster or variable rates of injection.

3. Similarly, the use of an intravenous rate of 100 mg. per kg. in 180 dogs, equal sexes, wide weight range (from 3 to 23 kg.), distributed among 6 groups of 30 dogs each, and with 5 per cent increases in dosage, gives an L D 50 of 58 mg. per kg. of amytal itself. This value agrees closely with the values reported by Swanson and Shonle<sup>32</sup> (1931) for a similar concentration and injection rate of pure sodium amytal.

4. Within the weight range of the rabbits, size played no rôle in the fatalities. In the dogs, the low range of from 2.7 to 5.4 kg. gave a higher mortality rate in each of the lower four dosages. However, by eliminating the extreme weights in various ways, the L D 50 in each case comes out 59 mg. per kg., or only 1 mg. higher than for the whole series.

5. No definite sex difference in regard to mortality is established, but a large scale study is suggested to test whether the female rabbit and dog may be a little more resistant than the male. In regard to body temperature and respiratory rate, the female rabbits were less depressed and on the whole recovered faster than the males.

6. No difference was found between mortalities in the warmer months of October and November as compared with the cooler months of December and January.

7. Microscopic examination of the liver and kidney with comparison of these tissues of supposedly normal dogs show that there is considerable pathologic change in our dogs and that no pathologic findings could be attributed to amytal.

8. Finally, a comparative dosage table for amytal has been compiled from the literature with addition of our own values and enough information added to make it possible for others better to judge the accuracy of each of the proposed dosages.

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## THE VELOCITY FACTOR IN BLOOD TRANSFUSION

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SINCE the time of the Egyptian kings man has tried to alleviate the suffering of his fellow men by introducing the blood of a healthy individual into the body of one who is ill.<sup>1</sup> This therapeutic procedure has persisted throughout the ages and has passed periods of acclaim and disrepute. The rationale of its use and the technic of its administration have varied from time to time. Our knowledge of transfusions has accumulated during these years. Many investigations have been made and pertinent facts have been ascertained, but the problem of transfusion reactions remains unsolved.

In studying the responses to intravenous injections, Hirshfeld et al.<sup>2</sup> have described a definite clinical syndrome which they term *Speed Shock*. This is characterized by an acute onset following the injection of a substance intravenously and giving rise to salivation, vomiting, diarrhea, dyspnea, muscle atony or spasm coincident with changes in the blood and in the circulatory and respiratory systems resulting in a fall in blood pressure and respiratory distress. They were unable to elicit this syndrome when the injections were made slowly and point out the danger of giving intravenous medication rapidly, and suggest that posttransfusion reactions are due to a rapid injection of blood, especially where a syringe method is employed. They suggest that for clinical purposes a rate of 2 or 3 c.c. per minute should be the upper limit for amounts of over 100 c.c., and 1 c.c. per minute for smaller quantities.

We have studied transfusions from the clinical aspect, maintaining a standard technic and utilizing only whole blood. While we could not control all possible variables in the clinical procedure as well as one might do in a laboratory test, because the life of a patient always was involved, we have utilized all the ordinary methods available to the clinician to enable a proper interpretation of our results. We wished to determine if velocity was responsible for any of our transfusion reactions.

### TECHNIC

Throughout this study we have used the Drummond Apparatus. This machine is essentially one of the stopcock type, designed to overcome the inconvenience and risk of error encountered in the use of apparatus employing a similar type valve (Fig. 1).

In this apparatus, as shown, it will be noted that the stopcock, which has three outlets, is mounted on the forward end of the machine. This moves in unison with the rotation of the barrel upon the plug, which is fixed. All air is

\*From the Philadelphia General Hospital.

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displaced from the lumen by physiologic saline solution and during transfusion a constant hydrostatic saline pressure is maintained upon the syringe while the barrel is in the level position. The normal donor to recipient flow cannot be reversed because of mechanical locks, while at any stage of the transfusion saline may be drawn into the syringe and given to the recipient or the lock on the donor's side may be released, if the operator desires, and an infusion of saline given the donor. By no means are you able to aspirate the recipient's vein at any time. Volume is accurately recorded by a calibrated disc which turns one marking each time the 5 c.c. syringe is emptied into the recipient's vein. This apparatus enabled us to use whole blood without the

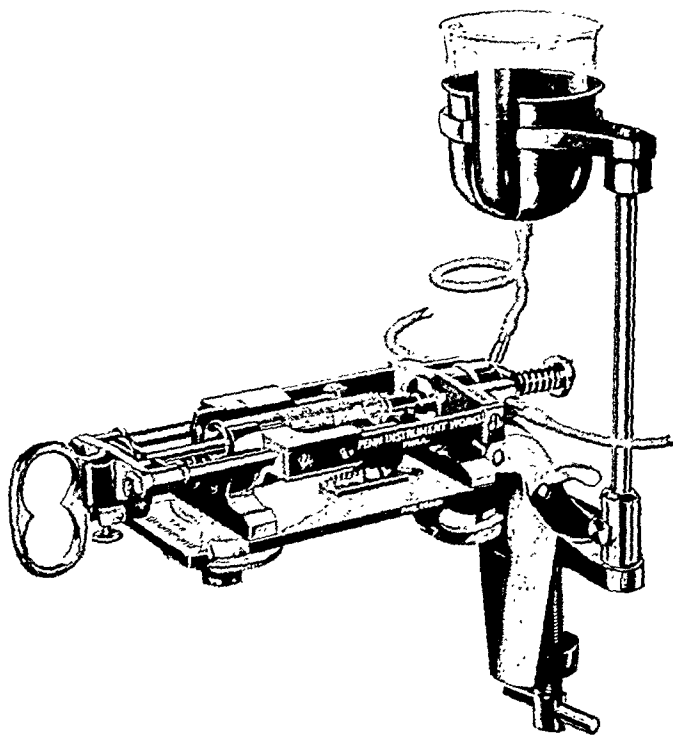


Fig. 1.

addition of citrate solution or any form of anticoagulant, and the ease of mechanical operation allowed us to give our entire attention to the reaction of the recipient.

All parts of the apparatus coming in contact with the blood were sterilized by boiling for twenty minutes. Other parts were immersed in alcohol or formalin solution for a like period. Our tubing was of an ordinary commercial type. We lubricated the steel piston with either sterile vaseline or a sterile citrated vaseline preparation and packed the open end of the syringe with the same material. This step in the preparation of the apparatus was demonstrated to us by Dr. John Noecker. It allows a thin film of grease to be spread over the inside of the glass syringe with each stroke of the piston and embodies the wax tube principle utilized by Kimpton Brown.<sup>10</sup> After

each transfusion we cleansed the apparatus with physiologic saline, hydrogen peroxide, and alcohol or ether.

We prefer that our donors always have a serologic examination, a typing, according to the Moss grouping,<sup>1</sup> and a cross-agglutination test with the recipient. We have found that the cross-agglutination test is never absolutely reliable unless observed for one hour. We know of no case in which we removed blood from a syphilitic donor.

A surgical technic was maintained in preparing the extremity for transfusion.<sup>12</sup> Tincture of iodine, 3½ per cent, and alcohol of 70 per cent strength were used on both anterior and posterior aspects of the area not covered by sterile drapes.

The pain incident to venipuncture was eliminated by a subcutaneous infiltration over the vessel to be tapped of a 1 per cent sterile novocaine solu-

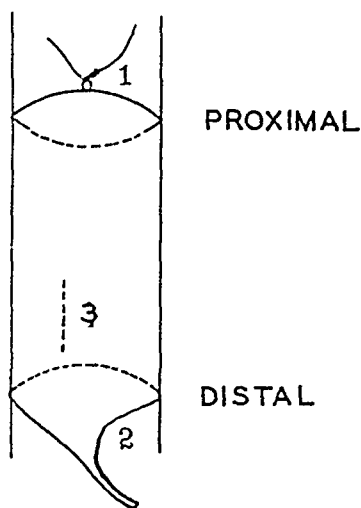


Fig. 2.—Showing vein held by suture material with single knot at 1 for retaining needle passed through incision at 3 and the blood current controlled by untied suture at 2.

tion. This procedure enabled the introduction of needles as large as 16 gauge without discomfort to either donor or recipient. When it was necessary to expose a vessel in order to introduce a needle into its lumen, we used novocaine anesthesia, making a small incision in the skin and carrying it down to the areolar tissue. Blunt dissection with a mosquito hemostat was used to isolate the vein, and it was brought to the skin surface by passing two short pieces of fine catgut under it. Separation of the ties, as shown in Fig. 2, enabled us to maintain complete control of the blood flow through the exposed section of the vessel. After the transfusion was completed and the retention ties removed from the vein it returned to its normal position. Temporary oozing was controlled by skin sutures and a pressure dressing.

Five minutes before giving the recipient the transfusion we recorded his blood pressure, temperature, pulse, and respiration, secured a complete blood count and a Schilling hemogram. Blood pressure was again recorded within five minutes after the transfusion and at the expiration of one hour, at which

time another blood count and Schilling hemogram were made. Urinalyses were made before and within twelve to twenty-four hours after transfusion. Temperature, pulse, and respiration were recorded at one, two, four, and six hours after transfusion. The following day another blood count and Schilling hemogram were secured.

The recipient's vein was tapped first. The needle being inserted in the direction of flow of the blood current. The efficiency of the venipuncture was tested by allowing at least 5 c.c. of the blood to flow into the syringe to which the needle was attached and immediately reinjecting this volume into the recipient's vein, his tourniquet having been released after the tap. We maintained the patency of the needle by injecting 10 to 15 c.c. of warm physiologic saline, during which time the tourniquet on the donor's arm was tightened and his vein tapped, the needle being inserted against the blood current. Connection with the apparatus was then completed, and with the appearance of blood in the syringe, from which it displaced physiologic saline, we considered the transfusion as started. Our estimation of total transfusion time includes from this instant until we withdrew the needle from the recipient's vessel. This was recorded by a stop watch.

The tolerance of the recipient to the blood was estimated clinically by giving the first 150 c.c. with extreme caution. We consider this a method of testing compatibility of bloods which should be regarded equally as important as the cross-agglutination test, providing the operator is trained in the clinical recognition of reactions. We always informed the patients of what was being done, when practicable, and asked them to tell us instantly of any new sensation or pain which they might experience. The facies, carotid pulsation and finger nails were observed for signs of cyanosis, distressed or altered expression, loss of pulse volume or acute circulatory collapse with resultant respiratory distress and embarrassment. These signs have been found clinically an earlier indicator of transfusion reaction than the substernal oppression, headache, and vomiting which all textbooks list.

Our estimation of velocity is based upon the total blood volume in cubic centimeters transfused, divided by the total transfusion time expressed in seconds. It is meant to accurately show the speed of transfer,<sup>9</sup> and is reported as cubic centimeters transfused per second. Velocity of transfusion depends upon the dexterity of the operator in temporarily connecting the donor's and recipient's veins. It is also influenced by the caliber of the needles used in the transfusion, it being impossible to propel in the same period of time, a given volume of blood through a needle of small caliber, as compared to one of larger lumen, providing we assume the propelling force to be the same. We have not attempted to approach the maximum in transfusion velocity but have maintained a standardized rate of transfusion which delivers the whole blood to the recipient in as near its natural condition as may be attained with present knowledge.

The danger of sudden death during transfusion is often totally absent from the operator's mind if the laboratory studies are favorable for transfusion. We have always kept prepared and ready for instant use a hypodermic of adrenalin and one of atropine sulphate.

## DISCUSSION

These cases were assembled from the routine work in the wards of the Philadelphia General Hospital. Our cases were not selected nor have we given any consideration, in recording our findings, to the metabolic, toxic or morbid state of the individual patient. We have studied a group of 37 patients who were given 50 transfusions. Thirty-nine transfusions consisted in the transference of ordinary whole blood; ten were of the nonspecific-immuno type<sup>4</sup> and one was a citrated nonspecific-immuno transfusion.

Our interpretation of a posttransfusion reaction was based upon the study of a composite clinical picture in each case. We considered a reaction as having occurred if the patient showed a sudden change of facial expression; cyanosis; complained of acute pain; had a loss of vascular tone or respiratory distress; suffered a chill with an acute rise in temperature; displayed a marked decrease in the number of circulating R.B.C. per c.mm.; and an associated increase in the Schilling hemogram "multiple index"\* (left shift) of 75 multiples; it having been shown by careful study that these changes in the clinical picture were not due to pathology present before the transfusion and unrecognized. Available material from our study leads us to believe that an increase in the multiple index of 75 constitutes the hemography of a posttransfusion reaction.

Kordenat and Smithies<sup>11</sup> found that in successful transfusions the post-transfusion leucocyte count was two or four times greater than before transfusion. Drinker and Brittingham<sup>8</sup> considered a rise in temperature of 2.5° F. as a reaction to the transfusion of citrated blood. We feel that transfusion reactions will be best interpreted when the decision is made, not upon a single clinical or laboratory factor, but is decided after a thorough study of the composite clinical picture of the individual case.

A total of 20,275 c.c. of whole blood was handled in this study, an average of 404 c.c. per transfusion. We considered the factor of acute cardiac dilatation only when the patient obviously had right heart failure. Dehydration is often present with the anemia, and before compatible blood could cause acute distention of a heart not already overburdened, the many venous lakes and the expansile factor resident in the venous side of the circulation would have to be taxed enormously. Crile has shown according to Col. C. Gordon Watson that the work of the heart increases in geometric ratio to the volume of blood, which in our transfusions was small. During the World War it was customary to transfuse large volumes of blood, usually 700 to 1,000 c.c. Major L. Bruce Robertson, C.A.M.C.<sup>7</sup> states that these cases never showed cardiac dilatation.

\*The "multiple index" indicates the number of times that a given shift is greater than normal. It is obtained by multiplying the Schilling index by 16. The normal Schilling index is 1/16. The normal "multiple index" is 1. The Schilling index is the ratio of shift cells (M, J, and St, the inefficient neutrophiles) to Sg or segmenters, the efficient neutrophiles.

$$\frac{M}{O} + \frac{J}{O} + \frac{St}{4} \cdot \frac{Sg}{64} = \frac{4}{64} \text{ or } \frac{1}{16}$$

If the Schilling index of an ill individual is  $\frac{3}{4}$ ,  $\frac{M}{2} + \frac{J}{4} + 30$ :

$$\frac{Sg}{48} = \frac{36}{48} = \frac{3}{4}$$

the "multiple index" is  $\frac{3}{4} \times 16 = 12$ . The Schilling index shows that there are 3 inefficient to every 4 efficient neutrophiles and the "multiple index" shows that the shift is 12 times greater than normal, that the Schilling index of  $\frac{3}{4}$  contains twelve 16ths, or 12 multiples of the normal left shift. Crocker and Valentine: Hemography in Diagnosis, Prognosis, and Treatment, J. LAB. & CLIN. MED. 1934. (In press.)

Our studies showed that:

*Average Velocity* of transfusion of whole blood was 1.02 c.c. per second.

*Blood Pressure.*—

Five minutes after transfusion, 45 comparisons:

|                               |                     |
|-------------------------------|---------------------|
| Systolic, increased 30 times  | average 12.1 mm. Hg |
| decreased 13 times            | average 14.0 mm. Hg |
| unchanged 2 times             |                     |
| Diastolic, increased 24 times | average 13.2 mm. Hg |
| decreased 14 times            | average 9.6 mm. Hg  |
| unchanged 7 times             |                     |

One hour after transfusion, 38 comparisons:

|                               |                     |
|-------------------------------|---------------------|
| Systolic, increased 20 times  | average 15.0 mm. Hg |
| decreased 15 times            | average 19.1 mm. Hg |
| unchanged 3 times             |                     |
| Diastolic, increased 18 times | average 13.8 mm. Hg |
| decreased 15 times            | average 12.6 mm. Hg |
| unchanged 5 times             |                     |

*W.B.C. Count.*—

One hour after transfusion, 50 comparisons:

|                    |                         |
|--------------------|-------------------------|
| increased 20 times | average 3,671 per c.mm. |
| decreased 30 times | average 3,205 per c.mm. |

Twelve to twenty-four hours after transfusion, 31 comparisons:

|                    |                           |
|--------------------|---------------------------|
| increased 12 times | average 5,120.8 per c.mm. |
| decreased 19 times | average 6,551.5 per c.mm. |

*R.B.C. Count.*—

One hour after transfusion, 50 comparisons:

|                    |                           |
|--------------------|---------------------------|
| increased 48 times | average 389,875 per c.mm. |
| decreased 2 times  | average 712,500 per c.mm. |

Twelve to twenty-four hours after transfusion, 31 comparisons:

|                    |                             |
|--------------------|-----------------------------|
| increased 21 times | average 509,523.8 per c.mm. |
| decreased 10 times | average 429,500.0 per c.mm. |

*Temperature.*—

Comparative readings at one, two, four, and six hours after transfusion, 38 comparisons:

|                    |                 |
|--------------------|-----------------|
| increased 33 times | average 1.3° F. |
| decreased 5 times  | average 2.8° F. |

*Pulse.*—

Comparative readings at one, two, four, and six hours after transfusion, 38 comparisons:

|                    |                                            |
|--------------------|--------------------------------------------|
| increased 29 times | average 141 $\frac{2}{3}$ beats per minute |
| decreased 9 times  | average 34 $\frac{2}{3}$ beats per minute  |

*Urinalyses.*—

Showed no changes of significance.

*Schilling Hemogram.*—

One hour after transfusion, 45 comparisons, of multiple index:

|                    |                                      |
|--------------------|--------------------------------------|
| increased 27 times | average 17.1 multiples (left shift)  |
| decreased 19 times | average 67.0 multiples (right shift) |

Twelve to twenty-four hours after transfusion, 29 comparisons, of multiple index:

|                    |                                      |
|--------------------|--------------------------------------|
| increased 12 times | average 37.8 multiples (left shift)  |
| decreased 17 times | average 73.8 multiples (right shift) |

*Pathologic Report.*—

Ultimate deaths

22

Autopsies

15

In no instance was death attributable to any other cause than the patient's disease. We have personally checked the histologic specimens of the kidneys in each of the fifteen autopsies and failed to find Bordley's post-transfusion kidney in any case.<sup>6</sup>

From an analysis of the composite clinical picture of posttransfusion reactions in our series of 50 transfusions, we found but two that showed evidence of a reaction. One was of a delayed type and one was of an acute nature. These two reactions represented 4 per cent of the total transfusions. The delayed reaction was due to the use of a so-called universal donor, without a cross-agglutination test having been made. The acute reaction, which conformed in every detail to Hirshfeld's description of *Speed Shock*, was found on recross-agglutination of the donor and recipient to be due to a definite incompatibility of the bloods which had either been missed at the previous examination or the titer of the agglutinating sera had been raised by the reaction.

Our observations to date include a series of over 125 transfusions of whole blood utilizing a similar technic. Two additional reactions have been noticed with the syndrome of *Speed Shock* in each case, but we have found that errors in compatibility were responsible for the reactions. There is a preponderance of opinion in the recent transfusion literature which emphasizes the fact that transfusion reactions are due to errors in compatibility (Parr and Krischner,<sup>3</sup> Polayes and Lederer,<sup>2</sup> Bordley,<sup>6</sup> Kordenat and Smithies,<sup>11</sup> also McClure and Dunn<sup>13</sup>).

While it has been held for years that the proper way to transfer blood was slowly, we have found that when the blood is compatible and untreated by extraneous chemicals, it may be given to the recipient according to our technic, at an average velocity of 1 c.c. per second without becoming a factor in the clinical picture of a posttransfusion reaction. We feel that keeping the blood out of contact with the vascular endothelium, which is its natural environment, for a prolonged period of time is undesirable.

#### CONCLUSIONS

1. The Drummond apparatus for blood transfusion is dependable and the mechanical principles which it embodies facilitate the transfusion of whole blood.

2. Anesthesia with a 1 per cent novocaine solution, as used in this study, eliminates the pain incident to venipuncture and should have a wider use in transfusion work.

3. The Schilling hemogram may be utilized in the study of transfusion reactions.

4. The *Speed Shock* syndrome was not elicited in the transfusion of whole blood at an average velocity of 1 c.c. per second except in cases where an incompatibility was demonstrated.

5. The study of a composite clinical picture is a preferable method of interpreting posttransfusion reactions.

6. Further studies on the velocity of transfusion of compatible whole blood are desirable.



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THE VALUE OF THE MONKEY FOR THE STUDY OF THE LAXATIVE  
ACTIVITY OF PHENOLPHTHALEIN, ESPECIALLY IN COMPAR-  
ING DIFFERENT SAMPLES OF THE DRUG\*

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MILLIONS of doses of phenolphthalein are taken annually in the United States for the well-known laxative action which it produces. For this purpose two general types of phenolphthalein, white and yellow (the more impure form), are placed on the market by manufacturers. The yellow product induces a much more powerful action than the white. Also, there is a considerable variation in the activity of samples of phenolphthalein from different manufacturers. Up to the present time the laxative activity of a particular sample could be estimated only by the use of human subjects, under constant conditions. A method of assaying this activity by the use of the monkey has been developed in the work described here.

All reports of the action of phenolphthalein in the lower animals fail to describe a laxative or purgative action similar to that in man except after relatively huge doses, usually requiring repetition. Such effects have been recorded by Abel and Rowntree<sup>1</sup> in dogs, van der Willigen<sup>2</sup> in cats, and Fleig<sup>3</sup> in dogs and rabbits. Vámosy<sup>4</sup> obtained no laxative action in dogs after a dose of 5 gm. and Fühner<sup>5</sup> found the drug completely inactive in mice after 20 mg. were given. On the other hand Loewe and Lange<sup>6</sup> reported a softening of the

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feces of mice after moderate doses (17 mg. per kilogram body weight), but failed to characterize their observation with a convincing description.

Fleig<sup>3</sup> expressed the situation admirably in the following statement: "It is to be regretted that an action, so intense in the case of man, hardly develops at all in the case of the animal and consequently cannot be studied with all the scientific precision of experimental analysis. Perhaps experiments performed on the monkey would be more fruitful; it would be interesting to try this animal." During the twenty-five years following this recommendation, no record of any one's having tried it has come to our attention.

#### EXPERIMENTAL

The samples of white and yellow phenolphthalein used in the experiments recorded here were made by the same manufacturer. The white was of U.S.P. grade and the yellow fulfilled all U.S.P. requirements except those concerning color. The latter possessed a very light canary yellow color in the powdered dry state, but in alcoholic solution showed a brownish yellow color modified by a slight fluorescence.

A series of 14 young white rats, weighing from 35 to 190 gm. each, received doses up to as much as 600 mg. (weight of rat, 135 gm.) of white and 84 mg. (weight of rat, 105 gm.) of yellow. Of the more than 500 doses given, only 2 (100 and 300 mg. of white phenolphthalein, respectively) were followed by a demonstrable softening of the feces. In both cases the rats failed to respond to a second and larger dose, furnishing further evidence of the inconstancy of the laxative action of phenolphthalein in the lower mammals.

A series of 5 cats, weighing from 2.2 to 4.0 kg., were given massive (5 to 10 gm.) doses of both white and yellow phenolphthalein in suspension in water or milk by stomach tube. Only repeated doses proved effective.

A dog, weighing 18 kg., showed no laxative action after a dose of 10 gm. of yellow phenolphthalein.

At this point we decided to try monkeys, although Fleig's suggestion had escaped our attention. These animals (*Macacus rhesus*), weighing from 2.0 to 3.5 kg., were found to be most suitable for the study of the drug. They were maintained on a regular diet of milk and eggs twice daily and fruits and vegetables once a day. Bone meal, cod liver oil, and at times blood meal were mixed into their fruit meal.

Each dose of phenolphthalein was accurately weighed on an analytical balance and then was quantitatively deposited in the interior of a piece of banana and sealed in. On feeding the piece of banana the monkey was closely watched to ascertain whether he consumed the material entirely. More pieces of banana then were fed to insure complete ingestion of the dose. If the quantity was sufficient to produce laxative action the effect would be evident in any case within from three to twenty-two hours, but usually within from six to twelve hours. After such a dose the stools became formless and semifluid, or if the dose was much above the minimum they became actually fluid. The former type of action properly is termed laxative, the latter purgative. When a minimum active dose was given, the action was always laxative and consisted of only a single action or of 2 or 3 at the most.

In order to establish the minimum therapeutic dose, gradually increasing quantities of the drug were given, with intervals of 1 mg. for doses less than 10 mg. and of 2 mg. for larger doses. Between each successive trial at least three full days were allowed to intervene. Finally that level was reached, below which no action occurred and above which laxative action always took place. This point has been determined for white phenolphthalein in 9 monkeys and for yellow in 5, as shown in Table I.

TABLE I  
MINIMUM THERAPEUTIC DOSES OF WHITE AND YELLOW PHENOLPHTHALEIN FOR MONKEYS

| MONKEY  | WHITE<br>PHENOLPHTHALEIN   | YELLOW<br>PHENOLPHTHALEIN  | RATIO: $\frac{\text{WHITE}}{\text{YELLOW}}$ |
|---------|----------------------------|----------------------------|---------------------------------------------|
|         | mg. per kg. body<br>weight | mg. per kg. body<br>weight |                                             |
| 1       | 2.8                        | 0.8                        | 3.7                                         |
| 1       |                            | 0.7                        | 2.3                                         |
| 2       | 6.8                        | 3.0                        |                                             |
| 3       | 4.8                        |                            |                                             |
| 4       | 5.0                        | 0.9                        | 4.5                                         |
| 4       |                            | 1.3                        |                                             |
| 7       | 4.0                        |                            |                                             |
| 8       | 4.1                        | 1.5                        | 2.7                                         |
| 9       | 2.1                        |                            |                                             |
| 9       | 2.1                        |                            |                                             |
| 10      | 5.9                        |                            |                                             |
| 10      | 5.7                        |                            |                                             |
| 11      | 4.1                        | 0.6                        | 6.8                                         |
| Average | 4.4                        | 1.4                        | 3.3                                         |

It will be noticed that the dose for 4 monkeys was redetermined with practically no variation from the level first established. The minimum dose for different animals, however, varied quite widely, as does the dose for human subjects. In one instance (Monkey 11) there is even a wide deviation from the average ratio of the dose of white phenolphthalein to that of yellow. As is usual in the case of experiments with animals, therefore, dependence should be placed rather in results obtained with several subjects than in those from a single animal.

The average doses thus obtained show the sample of yellow phenolphthalein to be about 3 times as powerful in laxative action as the white. This figure corresponds closely with the relative effect of these samples in human subjects. Other samples of phenolphthalein also have been assayed by using these same monkeys, and the results again confirmed the experience obtained with human subjects.

The field remains open for physiologic studies of the action of phenolphthalein on monkeys, with results which may enlighten us further as to its action on man.

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## THE FUNGICIDAL POWER OF PHENOL DERIVATIVES\*

### I. EFFECT OF INTRODUCING ALKYL GROUPS AND HALOGENS

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**S**IGNIFICANT of the unsatisfactory status of the present therapy of tinea infection is the large number of remedies used in its treatment. Considering the increasing incidence of the disease, its marked tendency to recur, and the frequency with which it persists in spite of prolonged treatment, comparatively few investigators have interested themselves in this particular problem of therapy. Apparently the first to do so were Bokorny<sup>1</sup> in Germany and Schamberg and Kolmer<sup>2</sup> in this country. Others whose publications have appeared at intervals in this field are Myers and Thienes,<sup>3</sup> Kingery and Adkisson,<sup>4</sup> Osborne and Hitchcock,<sup>5</sup> Schamberg, Brown and Harkins,<sup>6</sup> Bonar and Dreyer,<sup>7</sup> Smyth and Smyth,<sup>8</sup> and Legge, Bonar and Templeton.<sup>9</sup> These studies indicated that such phenol derivatives as thymol and salicylic acid which have long been known for their clinical usefulness have a just claim to their popularity. They also indicated that there might be other phenol derivatives less irritating than thymol but more effective toward fungi.

In recent years numerous studies of the *germicidal* properties of alkyl phenol derivatives have been made. Included in these is the work of Laubenthal,<sup>10</sup> Johnson, Hodge and Lane,<sup>11</sup> Schaffer and Tilley,<sup>12</sup> Klarman and his collaborators,<sup>13</sup> Rettger, Plastridge and Valley,<sup>14</sup> Coulthard, Marshall and Pyman,<sup>15</sup> Read and Miller<sup>16</sup> and others.<sup>17, 18, 19, 20, 21</sup> Substitution of halogens in phenols has been studied by Koch,<sup>22</sup> Bechold and Ehrlich,<sup>23</sup> and Klarman and Von Wövern.<sup>24</sup>

The present work was done in an attempt to follow up the work of Kingery and Adkisson<sup>4</sup> and find, if possible, derivatives of phenol which might be used internally as therapeutic agents. As a first step it was thought desirable to learn the effects on fungicidal properties produced by the introduction of various types of groups into phenol.

*Test Organisms.*—The entire group of cultures used in this work was obtained through the kindness of Dr. Fred Weidman of the University of Penn-

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sylvania Medical School. The group was composed of the following fourteen yeasts and molds, practically all of which are known to be of a pathogenic nature—*Monilia tropicalis*, *Sporotrichum*, *Cephalosporium*, *Microsporon fulvum*, *Trichophyton interdigitale*, *Blastomyces*, *Monilia candida*, *Monilia pinoyi*, *Monilia metalondinensis*, *Monilia macedoniensis*, *Penicillium brevicaulis*, *Cryptococcus histolyticus*, *Coccidioides immitis*, *Trichophyton purpureum*.

*Compounds Tested.*—Thirty-seven derivatives of phenol were tested with regard to their fungicidal power. Phenol, itself, was used as a basis for comparison. We are indebted to Dr. E. Klarmann of the Plaut Research Laboratory of Lehn and Fink for his kindness in furnishing samples of the four ethers of resorcinol listed in the table. A few of the compounds were prepared in this laboratory. Included in this group were 3, 5-diethyl phenol; 3, 5-dibutyl phenol, n-amyl salicylate; and 2, 4-dinitrophenol. The m-cresol and p-chlorophenol were purified before use. For purposes of comparison two other common fungicides, iodine and mercuric chloride, were also tested.

#### EXPERIMENTAL

*Method of Testing.*—The method used by Kingery and Adkisson<sup>4</sup> in their fungicidal tests has been followed with certain minor changes. Sabouraud's dextrose agar was used for the solid media. This was prepared by adding 10 gm. "Difco"-Bacto peptone, 40 gm. crude glucose, and 15 gm. agar to 1,000 c.c. tap water. After the media had been adjusted to a pH of 5.2 to 5.4, it was autoclaved for twenty minutes at fifteen pounds pressure. The liquid medium used was dextrose broth adjusted and prepared in the same manner with the omission of the agar. Stock cultures were grown on the solid media.

Culture tubes containing from 10 to 15 c.c. of broth were planted with a loopful of yeast from the stock tubes, thoroughly mixed and grown, seventy-two hours at 37.5° C. in case of the yeasts and longer at room temperature in case of the molds.

The compound, the fungicidal power of which was to be determined, was dissolved in water whenever possible and diluted to the desired concentration. In a few cases it was necessary to use dilute solutions of alcohol, glycerol, sodium carbonate or potassium hydroxide as solvents.

To 2 c.c. of the drug solution contained in a sterile test tube was added 1 c.c. of the broth suspension of the yeast or mold.

The mixture was then shaken and at the end of one minute, thirty minutes, and sixty minutes several loopfuls were transferred to separate sections of a plate of Sabouraud's dextrose agar. The plates had previously been divided into quarters by marking on the bottom of the Petri dishes with a wax pencil. The fourth quarter was used as a control. Transplants were made to this section from a tube containing 2 c.c. of sterile distilled water and 1 c.c. of yeast suspension.

The plate was then incubated for a period of time depending upon the organism being tested. The yeasts required seventy-two hours at 37.5° C. while it was found that the molds gave the best results when left at room temperature at least a week. All plates were left at room temperature for three weeks when final readings were taken.

All tests were made at room temperature which varied from 19° C. to 23° C.

*Choice of Three Organisms.*—Because of the large amount of work involved in the preparation of cultures it was found necessary to limit the number of organisms used.

Preliminary tests made with the entire group showed that three organisms, *Monilia tropicalis*, *Sporotrichum*, and *Cephalosporium*, were the most representative. *Monilia tropicalis* has the appearance of a typical yeast and shows vigorous growth in the incubator. *Sporotrichum* seems to lie between the yeasts and molds in form while *Cephalosporium* is more nearly a true mold. The latter two organisms grow quite vigorously at room temperature. All three show more than average resistance to the fungicidal agents tested: All compounds were tested against *M. tropicalis* which showed the highest resistance in all cases, while *Sporotrichum* and *Cephalosporium* were used as checks with all of the more effective compounds but not with the simpler phenols.

*Control of Environmental Factors.*—*Temperature.* Since in the case of bactericidal studies an increase in temperature has been reported to cause a marked increase in the germicidal strength of various drugs, a few tests were carried out to determine to what extent the working temperature should be controlled for satisfactory results in fungicidal studies.

An increase in temperature from 10° to 30° C. was found to increase the fungicidal ability of the drug slightly. However, the difference between the effect at 21° and that at 30° C. was small enough to make it seem unnecessary to take any special precautions to control the room temperature while making the tests.

*Solubility.*—The most troublesome factor which had to be contended with throughout the work was the near insolubility of a large number of the compounds. An effort was made in every case to obtain an aqueous solution of the drug to eliminate any possible fungicidal action of the solvent. However, it was found necessary in a few cases to use 10 per cent alcohol, 1 per cent sodium carbonate, 30 per cent glycerol, and 0.5 per cent potassium hydroxide solutions as solvents. None of these solvents themselves were found to kill the organisms under the conditions of our experiments.

It has been suggested that the insolubility of compounds may be closely connected with their ability as antiparasitic agents.<sup>20</sup> When a drug which is highly insoluble in water comes into contact with the lipoids of the cells in which it is more soluble, a rapid absorption of the drug by the cell is thought to result. All of the compounds which have been tested in this work and found to be highly toxic to yeast and molds are very slightly soluble in water. It was found that a water soluble, colloidal trade preparation of chlorothymol known as "elymocol" was not as effective a fungicidal agent as the crystalline chlorothymol.

While using 1 per cent sodium carbonate solution as a solvent, it was found that the fungicidal power of a compound dissolved in the carbonate solution was less than that of the same compound in an aqueous solution. Salicylic acid dissolved in water at a dilution of 1:1500 is more effective as

a fungicide than salicylic acid dissolved in 1 per cent sodium carbonate at a dilution of 1-500 or sodium salicylate dissolved in a 1-500 aqueous solution. On the other hand, thymol, carvacrol and chlorothymol are only slightly less effective in a sodium carbonate solution. Compounds with stronger acid properties are apparently more affected by being used in an alkaline medium than more weakly acidic substances.

*Hydrogen Ion Concentration.*—By use of a quinhydrone electrode the pH of the dextrose broth was found to be 5.3. The 1 per cent  $\text{Na}_2\text{CO}_3$  solution had a pH of about 9 as did also a mixture of 1 c.c. of broth and 2 c.c. of 1 per cent  $\text{Na}_2\text{CO}_3$  solution.

It has been indicated before that the sodium carbonate solution did not prevent the subsequent growth of the yeast and molds. It is evident, therefore, that a marked shift in the hydrogen ion concentration toward the basic end of the scale does not produce a fungicidal action.

*Results of Fungicidal Tests.*—Table I summarizes the results of the fungicidal tests made on the three organisms selected. The maximum dilution of each compound effective at thirty minutes and the phenol coefficient calculated by using the thirty-minute tests as a basis are given for each organism. The phenol coefficients were calculated by dividing the maximum effective dilution of a compound at thirty minutes by the maximum effective dilution of phenol at thirty minutes when using the same organism. Where not enough tests were made with a compound to make possible the determination of a definite coefficient, an approximate coefficient is indicated.

#### DISCUSSION

It may be seen from the table that certain structural features are necessary in a compound for it to possess high fungicidal power. The substitution of one or more alkyl groups on the benzene ring in phenols increases the fungicidal power, the resulting phenol coefficient depending upon the number of carbon atoms in the side chain. Compounds containing an alkyl group with six carbon atoms arranged in a straight chain have the highest coefficients as far as it is possible to tell from present results. The position in the ring occupied by the substituted alkyl groups makes no striking difference although the work has shown that the meta and para compounds are slightly stronger in fungicidal power.

Dialkyl compounds are stronger in fungicidal power than the corresponding monoalkyl compounds. One would anticipate that a dihexyl phenol would have a very high fungicidal ability except that its use is probably prevented by its insolubility. In the case of the dihydric phenols it has been shown that it matters not whether the alkyl group is substituted for a hydrogen atom of the benzene ring or a hydrogen atom of the hydroxyl group. The isomeric compounds, *n*-hexyl resorcinol and the *n*-hexyl ether of resorcinol, have coefficients which are nearly identical.

The indications are, from the five compounds studied, that the introduction of one or more nitro groups into phenols does not increase the fungicidal power materially.

TABLE I  
FUNGICIDAL TESTS ON SUBSTITUTED PHENOLS  
(Time: Thirty Minutes)

| COMPOUNDS                                                        | <i>M. Tropicalis</i> |                  | <i>Cephalosporium</i> |                  | <i>Sporotrichum</i> |                  |
|------------------------------------------------------------------|----------------------|------------------|-----------------------|------------------|---------------------|------------------|
|                                                                  | EFFECT.<br>DILN.     | PHENOL<br>COEFF. | EFFECT.<br>DILN.      | PHENOL<br>COEFF. | EFFECT.<br>DILN.    | PHENOL<br>COEFF. |
| 1. Phenol                                                        | 1-75                 | 1.0              | 1-110                 | 1.0              | 1-150               | 1.0              |
| 2. o-Cresol                                                      | 1-175                | 2.3              | -----                 | -----            | -----               | -----            |
| 3. p-Cresol                                                      | 1-190                | 2.5              | -----                 | -----            | -----               | -----            |
| 4. m-Cresol                                                      | 1-200                | 2.6              | -----                 | -----            | -----               | -----            |
| 5. 4-OH-1, 2 Dimethyl benzene                                    | 1-500                | 6.6              | -----                 | -----            | -----               | -----            |
| 6. 4-OH-1, 3 Dimethyl benzene                                    | 1-475                | 6.3              | -----                 | -----            | -----               | -----            |
| 7. 2-OH-1, 3 Dimethyl benzene                                    | 1-400                | 5.3              | -----                 | -----            | -----               | -----            |
| 8. 2-OH-1, 4 Dimethyl benzene                                    | 1-350                | 4.7              | -----                 | -----            | -----               | -----            |
| 9. 3, 5-Diethyl Phenol (0.5% KOH)                                | 1-2,000              | 26.6             | 1-2,000               | 18.2             | 1-2,500             | 16.6             |
| 10. 3, 5-Dibutyl Phenol (1% Na <sub>2</sub> CO <sub>3</sub> )    | 1-±11,000            | ±160.0           | -----                 | -----            | -----               | -----            |
| 11. Thymol                                                       | 1-2,000              | 26.6             | 1-3,000               | 27.3             | 1-3,000             | 20.0             |
| 12. Carvacrol                                                    | 1-2,000              | 26.6             | 1-2,500               | 22.7             | 1-3,000             | 20.0             |
| 13. o-Nitrophenol                                                | 1-±500               | ±6.6             | -----                 | -----            | -----               | -----            |
| 14. m-Nitrophenol                                                | 1-±400               | ±5.3             | -----                 | -----            | -----               | -----            |
| 15. p-Nitrophenol                                                | 1-±400               | ±5.3             | -----                 | -----            | -----               | -----            |
| 16. 2, 4-Dinitrophenol (1% Na <sub>2</sub> CO <sub>3</sub> )     | 1-±200               | ±2.6             | -----                 | -----            | -----               | -----            |
| 17. 2, 4, 6-Trinitrophenol (1% Na <sub>2</sub> CO <sub>3</sub> ) | 1-±400               | ±5.3             | -----                 | -----            | -----               | -----            |
| 18. o-Chlorophenol                                               | 1-275                | 3.7              | -----                 | -----            | -----               | -----            |
| 19. m-Chlorophenol                                               | 1-300                | 4.0              | -----                 | -----            | -----               | -----            |
| 20. p-Chlorophenol                                               | 1-300                | 4.0              | -----                 | -----            | -----               | -----            |
| 21. o-Bromophenol                                                | 1-500                | 6.6              | -----                 | -----            | -----               | -----            |
| 22. p-Bromophenol                                                | 1-500                | 6.6              | -----                 | -----            | -----               | -----            |
| 23. o-Iodophenol                                                 | 1-750                | 10.0             | -----                 | -----            | -----               | -----            |
| 24. o-Hydroxy benzoic acid                                       | 1-1,000              | 13.3             | 1-2,000               | 18.2             | 1-2,000             | 13.3             |
| 25. m-Hydroxy benzoic acid                                       | 1-±500               | ±6.6             | -----                 | -----            | -----               | -----            |
| 26. p-Hydroxy benzoic acid                                       | 1-±500               | ±6.6             | -----                 | -----            | -----               | -----            |
| 27. Sodium salicylate                                            | 1-500                | 6.6              | -----                 | -----            | -----               | -----            |
| 28. n-Amyl salicylate (20% alcohol)                              | 1-±4,000             | ±53.0            | -----                 | -----            | -----               | -----            |
| 29. Catechol                                                     | 1-50                 | 0.66             | -----                 | -----            | -----               | -----            |
| 30. Resorcinol                                                   | 1-25                 | 0.33             | -----                 | -----            | -----               | -----            |
| 31. Hydroquinone                                                 | 1-25                 | 0.33             | -----                 | -----            | -----               | -----            |
| 32. n-Hexyl resorcinol (30% glycerine)                           | 1-15,000             | 200.0            | 1-17,000              | 154.0            | 1-20,000            | 133.0            |
| 33. n-Butyl ether of resorcinol                                  | 1-1,500              | 20.0             | 1-2,000               | 18.0             | 1-4,000             | 26.6             |
| 34. n-Amyl ether of resorcinol                                   | 1-4,500              | 60.0             | 1,7,000               | 63.6             | 1-9,000             | 60.0             |
| 35. n-Hexyl ether of resorcinol                                  | 1-11,000             | 146.0            | 1-17,000              | 154.0            | 1-22,000            | 146.0            |
| 36. Phenyl propyl ether of resorcinol                            | 1-7,500              | 100.0            | -----                 | -----            | -----               | -----            |
| 37. Chlorothymol                                                 | 1-8,000              | 106.6            | 1-16,000              | 146.0            | 1-20,000            | 133.0            |
| 38. "Clymocol"                                                   | 1-±5,000             | ±66.0            | 1-±5,000              | ±45.0            | 1-±5,000            | ±33.0            |
| 39. 2-Cl-5-OH-toluene                                            | 1-1,000              | 13.3             | 1-1,500               | 13.6             | 1-2,000             | 13.3             |
| 40. Iodine                                                       | 1-20,000             | 266.0            | 1-20,000              | 182.0            | 1-30,000            | 200.0            |
| 41. Mercuric chloride                                            | 1-±20,000            | 266.0            | -----                 | -----            | -----               | -----            |

± "Approximate"; insolubility of the drug made more accurate estimation impossible.

The substitution of halogen atoms in phenols increases their fungicidal ability several times. It is worth noting that both in the case of phenol and thymol, the introduction of a chlorine atom increases the fungicidal power the same amount, namely fourfold. A substituted iodine atom has a greater effect than a bromine atom which in turn produces a higher phenol coefficient than does a chlorine atom. The substitution of halogen atoms also decreases the solubility of the compounds. Again the position on the ring taken by the



substituted halogen atom has no marked effect upon the fungicidal power.

In contrast to the statement that the position occupied by substituted alkyl groups or halogen atoms is unimportant is the fact that a carboxyl group substituted in the ortho position has been shown to exert an effect more than twice as great as when substituted in the meta or para positions. The substitution of the carboxyl group in the ortho position increases the fungicidal power of phenol more than does a halogen atom or short alkyl side chain.

Of the phenol derivatives which have been studied n-hexyl resorcinol and the n-hexyl ether of resorcinol possess the highest fungicidal power followed closely by chlorothymol. All three should prove useful in the treatment of fungous skin infections and all are being subjected to clinical trial. The n-butyl, n-amyl and phenyl propyl ethers of resorcinol as well as 3,5-dibutyl phenol have exhibited sufficient fungicidal ability also to warrant further study. Thymol, carvacrol and salicylic acid are now being used as fungicides and have given fairly satisfactory results in the actual treatment of skin infections.

*Fungicidal Power Compared with Bactericidal Power.*—A close relationship between fungicidal and bactericidal power has been observed throughout this study of phenol derivatives. The highest dilution of phenol which will kill *M. tropicalis*, (1-75) is very nearly the same as the maximum dilution (1-80) which kills *B. typhosus*. The dihydric phenols, hydroquinone, resorcinol and catechol have coefficients of less than one on both bacteria (*B. typhosus* and *Staph. aureus*)<sup>13</sup> and fungous organisms (*M. tropicalis*). The coefficient (2.5) for p-cresol (*B. typhosus*) reported by Coulthard, Marshall and Pyman<sup>15</sup> compares with the coefficient of 2.5 obtained when using *M. tropicalis*. As the length of the side chain increases, in alkyl substituted phenols, however, the phenol coefficients increase but more rapidly if the tests are made with fungous organisms than when bacteria are used. Thus hexyl resorcinol has a coefficient of 200 with *M. tropicalis*, 154 with *Cephalosporium*, and 133 with *Sporotrichum*, all fungous organisms, while the commonly accepted coefficient for the same substance on *B. typhosus* is approximately 46. This difference may be due in part to the use of a different method of calculating the coefficients. An interesting comparison may be made between the results obtained by Klarmann<sup>13</sup> and those obtained in this work with the monoethers of resorcinol.

TABLE II  
PHENOL COEFFICIENTS

|                             | <i>B.<br/>Typhosus</i><br>(REPORTED BY KLARMANN) | <i>Staph.<br/>Aureus</i> | <i>M.<br/>Tropicalis</i> | <i>Cephal-<br/>osporium</i> | <i>Sporo-<br/>trichum</i> |
|-----------------------------|--------------------------------------------------|--------------------------|--------------------------|-----------------------------|---------------------------|
| n-butyl ether of resorcinol | 20                                               | 18                       | 20                       | 18                          | 26.6                      |
| n-amyl ether of resorcinol  | 35                                               | 36                       | 60                       | 64                          | 60                        |
| n-hexyl ether of resorcinol | 46                                               | 125                      | 146                      | 154                         | 146                       |

#### SUMMARY

1. The fungicidal powers of 37 derivatives of phenol and of two miscellaneous fungicides, iodine and mercuric chloride, have been determined by

using as test organisms *Monilia tropicalis*, *Cephalosporium*, and *Sporotrichum*. These organisms were selected after preliminary tests as being typical of a large group of pathogenic yeasts and molds.

2. The position taken by the substituted alkyl side chains and halogen atoms has been found in general to have little effect on the fungicidal power of phenols; on the other hand salicylic acid, a phenol derivative having a carboxyl group in the ortho position, is far superior as a fungicide to the isomeric meta- and para hydroxy benzoic acids.

3. It has been shown that there is a definite increase in the fungicidal power of the alkyl phenol derivatives with an increase in the number of carbon atoms of the side chain; that in dihydric phenols the substituted alkyl radical may take the place of a hydrogen atom of the benzene ring or of a hydrogen atom of the hydroxyl group without causing any significant variation in the fungicidal power.

4. The substitution of halogen atoms has been found to increase the fungicidal power from four to ten times; the fungicidal strength increasing from chlorine through bromine to iodine. The introduction of nitro groups into phenols does not increase the fungicidal power.

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# LABORATORY METHODS

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## OBSERVATIONS ON THE TECHNIC OF THE FRIEDMAN TEST FOR PREGNANCY\*

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IN A RECENT address Dr. T. B. Magath<sup>1</sup> editor of the *American Journal of Clinical Pathology*, made the following statement: "Although clinicians have scientifically studied the signs of pregnancy for more than 5,000 years, recently devised simple laboratory tests can detect pregnancy earlier and more accurately than any set of clinical observations yet perfected."

The first practical laboratory test for pregnancy was introduced in 1927 by Aschheim and Zondek.<sup>2</sup> It consists of six subcutaneous injections of urine twice daily for three days into five immature mice, followed by inspection of the animals' ovaries one hundred hours after the first injection. The presence of blood points and lutein tissue constitutes a positive reaction. Aschheim and Zondek reported a series of 1,400 tests with 98.5 per cent accuracy. The test has now been performed throughout the world in many thousand cases, and its reliability fully established. The material in the urine responsible for the reaction was called prolان and was considered to consist of two anterior pituitary hormones.

The hypophyseal principle was found by Zondek in the hypophysis, in certain extracts of other organs, in placental tissue, decidual tissue and perhaps most important of all in the urine of pregnant women. A small quantity (about one mouse unit) was found in the urine of nonpregnant females and as much as from 3,000 to 5,000 units in pregnant women. This observation led Aschheim and Zondek<sup>3</sup> to propose the demonstration of a large quantity of prolان in the urine as a test for pregnancy. Although there is some question as to the site of origin and nature of prolان, the evidence seems to favor the view that it is an anterior hypophyseal secretion and that because of the presence of the living placental tissue in the pregnant woman it is formed continuously in very large amounts and excreted in part in the urine.

The pregnancy test was the outcome of animal experiments by Zondek<sup>3</sup> in the study of ovarian function. These studies of Zondek in Germany and those of P. E. Smith<sup>4</sup> in this country in 1926 were the first to reveal that sexual maturity was controlled by the anterior hypophysis and that hormones from this body initiate follicle ripening, ovulation and luteinization.

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In 1929, Friedman<sup>3</sup> recommended the use of a single mature rabbit to replace the five immature mice of the Aschheim-Zondek test. In the rabbit there is no periodic ovulation or luteinization of follicles and these changes normally occur only after copulation, consequently the mature female rabbit isolated from the male for a period of several weeks is an ideal animal for demonstrating the effect of prolactin. In addition to the simplification of the pregnancy test by the use of one rabbit, the time required is shortened from one hundred hours to forty-eight hours. The technique suggested by Friedman<sup>3</sup> in 1931 consists in the intravenous injection of 4 c.c. of a voided specimen of urine (three times daily for two days), a total of 24 c.c., into a mature female rabbit previously isolated for at least three weeks. About forty-eight hours after the first injection the ovaries are examined at autopsy. In a positive test the ovaries are enlarged, and show a number of prominent hemorrhagic follicles. In a negative test the ovaries are white, and while ripe follicles 1 to 2 mm. in diameter may be seen these contain clear colorless fluid.

Since the original publication by Friedman<sup>3</sup> a large number of tests for pregnancy, using rabbits as the test animal, have been reported in this country<sup>6, 7</sup> confirming his results.

The results of the 350 tests performed at the Mount Sinai Hospital of Cleveland are shown in Table I.

The earliest positive test in this series was thirty-two days after the last menstrual period.

TABLE I

## SUMMARY OF 350 FRIEDMAN TESTS AT MOUNT SINAI HOSPITAL OF CLEVELAND

|                                               |                |
|-----------------------------------------------|----------------|
| Total tests                                   | 350            |
| True positives (uterine pregnancy)            | 92             |
| True positives                                |                |
| (intact ectopic pregnancy)                    | 2              |
| (hydatid mole)                                | 1              |
| False positives (tumors)                      | 1 + 2 in males |
| False positives (tuberculous oophoritis)      | 1              |
| True negatives                                | 131            |
| Not pregnant                                  | 69             |
| Delayed menses                                | 12             |
| Fibromyoma uterus                             | 10             |
| Acute pelvic inflammatory disease             | 1              |
| Chronic pelvic inflammatory disease           | 1              |
| Tuberculous pelvic peritonitis                | 10             |
| Carcinoma cervix                              | 7              |
| Ovarian cyst                                  | 4              |
| Subacute endometritis                         | 3              |
| Postpartum                                    | 2              |
| Menopause                                     | 2              |
| Ectopic pregnancy (no intact chorionic villi) | 2              |
| Polypoid endometrium                          | 1              |
| Hydatid mole (postoperative)                  | 1              |
| Carcinoma breast                              | 1              |
| Carcinoma ovary                               | 1              |
| Benign tumor of serotum                       | 1              |
| Sarcoma of uterus                             | 1              |
| Fibromyoma with sarcomatous change            | 1              |
| Male urine (200 c.c.)                         | 1              |
| False negatives                               | 1              |
| Doubtful (early tests of series)              | 5              |
| Experimental                                  | 114            |

The one false positive and the one false negative test will be discussed later. Tests were done in four cases of tubal pregnancy, two of which were positive and two negative. On microscopic examination of the fallopian tubes intact chorionic villi were present in both cases which were positive while in the negative cases the villi were degenerating or necrotic.

In the case of hydatid mole the preoperative specimen of urine gave a characteristic strongly positive reaction and a negative result was obtained on testing the urine twelve days postoperatively. In the cases of tumors other than hydatid mole three gave positive results and twenty-two gave negative results. Of the three positive tests one was a female with a clinical diagnosis of teratoma of the ovary and the other two tests were in the case of a male with a clinical diagnosis of teratoma of the testis. The urines from the cases of teratoma of the ovary and of the testis were obtained through the courtesy of Dr. D. Seecof of City Hospital, Cleveland. The negative tests included various types of benign and malignant genital tumors as shown in Table I.

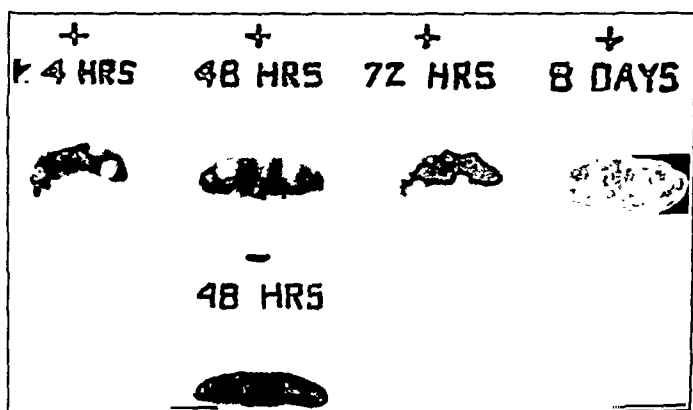


Fig. 1.—Gross photograph. Natural size. Rabbit ovaries. Gross appearance of positive test of one, two, three and eight days' duration and of negative test.

#### OBSERVATIONS RELATING TO THE TECHNIC OF THE FRIEDMAN TEST

Many minor modifications of the technic of the Friedman test have already been reported in the literature.

*A. Selection of Animals.*—Of first importance is the selection of proper animals. Some workers have used animals from three to four months of age. Friedman<sup>6</sup> recommends the use of rabbits after bearing young for biologic assay, as these animals give the most constant response to a given dose of the hormone. For routine use, however, ordinary domestic female rabbits weighing from 2 to 4 kg. give satisfactory results.

As in other biologic tests the rabbits are found to vary considerably in their reaction to a given dose of hormone. In a series of 55 mature rabbits, each of which was injected with 10 c.c. of pooled urine from pregnant women, 49 gave characteristic positive reactions, 4 gave minimal or grossly doubtful reactions, and 2 were negative. One of the two negatively reacting rabbits gave a negative result on repeating the test with a 10 c.c. dose but a positive result on injecting 20 c.c. The other was not retested.

As stated above the rabbits must be isolated from male rabbits or other females for a period of three weeks before injection. Some workers have eliminated this period of isolation by preliminary laparotomy and selection of those animals that have obviously not experienced hypophyseal stimulation for several weeks, with ovaries showing ripe or nearly ripe follicles with clear fluid but no hemorrhagic areas or corpora lutea.

The importance of inspection of the ovaries before selection of the animals was emphasized by Wood<sup>9</sup> who states: "We attach much importance to the preliminary inspection of the ovaries as we feel that this gives us an ideal opportunity for correctly interpreting the actual changes brought about by the anterior pituitary hormone present in the urine. This procedure is in our judgment the best possible control of the test."

We have followed this suggestion in 178 tests and recommend its use as strongly as does Wood.

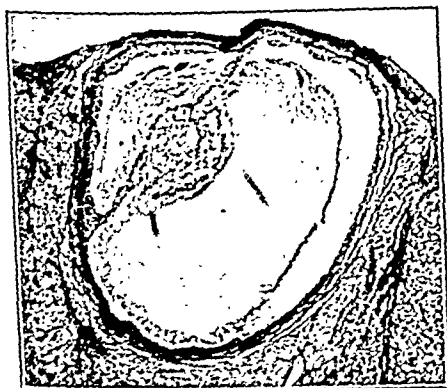


Fig. 2.



Fig. 3.

Fig. 2.—Photomicrograph. Low power. Rabbit ovary. Normal ripe follicle.

Fig. 3.—Photomicrograph. High power. Rabbit ovary. Normal ripe follicle, showing granulosa cells and theca.

Of the 178 rabbits in which preliminary inspection of the ovaries was done, three showed corpora hemorrhagica, seven showed corpora lutea, four showed old areas of hemorrhage, and seven were found to be pregnant. These findings show the necessity of isolating rabbits several weeks or more for the complete involution of corpora lutea before the animal is in a satisfactory condition for the test. In the case of the seven rabbits (recent purchases) which were found to be pregnant on preliminary inspection, the test would have had to be repeated as Friedman has shown that, during the pregnancy, the rabbit requires a larger amount of the hormone to produce corpora hemorrhagica than it does in the non-pregnant state.

Of the 3 rabbits showing corpora hemorrhagica, one had not been used previously and had been isolated for a period of three weeks, the other 2 had been positive about four weeks previously. Of the 7 rabbits showing corpora lutea, 4 were recent purchases, isolated about one week, the other 3 had been

positive from one to two months previously. Of the four rabbits showing old areas of hemorrhage one was recently purchased and isolated only several days, the other three had given positive results, from one to two and one-half months previously.

It will be noted from the above figures that the majority of corpora hemorrhagica and old areas of hemorrhage (the changes which would lead to false positive results) observed from three to ten weeks after hypophyseal stimulation occurred in animals which had been used previously and in which positive results were obtained. In our experience 4 out of 5 rabbits as purchased in the open market were satisfactory upon preliminary inspection for immediate use in the Friedman test. In those as purchased on the market and isolated for the prescribed period of three weeks only one showed a corpus hemorrhagicum and

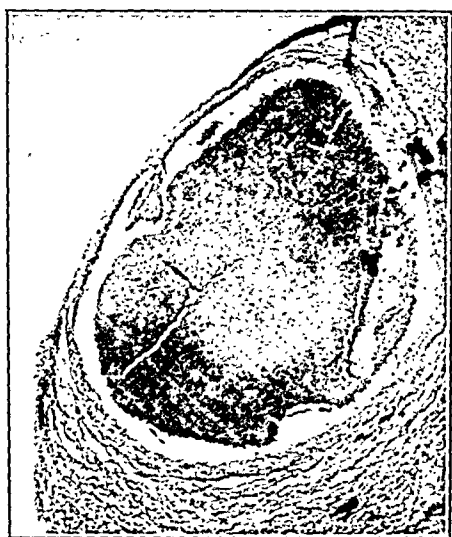


Fig. 4.

Fig. 4.—Photomicrograph. High power. Rabbit ovary. Positive test of twenty-four hours' duration (early corpus hemorrhagicum).



Fig. 5.

Fig. 5.—Photomicrograph. Low power. Rabbit ovary. Positive test of forty-eight hours' duration (early corpus luteum).

might have given rise to a false positive result. The preliminary operation, therefore, is of particular value in animals previously giving positive results, the period of complete involution in these artificially produced corpora hemorrhagica being variable and in some instances requiring longer than three weeks (see Fig. 10 of subinvolved ovary).

All of the rabbits were isolated from other rabbits as soon as purchased and were kept in individual pens at all times.

**B. Anesthesia.**—The anesthetic generally employed for laparotomy has been ether, although the use of sodium barbital and avertin<sup>10</sup> and other anesthetics has been reported. In the series of tests reported here morphine sulphate was injected intravenously and found most satisfactory. While the majority of tests were done using a half grain of morphine for rabbits weighing from 2 to 3½ kg.



(the average size of the animals used in the test) doses as high as 2 gr. were employed in the early tests of this series. Within a few minutes after the injection of the morphine, the animal becomes drowsy and relaxed and is ready for operation. Rabbits anesthetized with morphine will show local reflexes with muscular contraction on sharp cutting and quick handling but no pain apparently. These reflexes can be minimized or even abolished if the movements of cutting and handling are deliberate. Rarely the larger animals are insufficiently anesthetized with a half grain of morphine, whereupon an additional quarter grain is given. The animals remain drowsy for several hours and at no time do they show any ill effects. The use of morphine intravenously has the additional advantage of making it possible to perform the laparotomy without an assistant and of being economical.



Fig. 6.

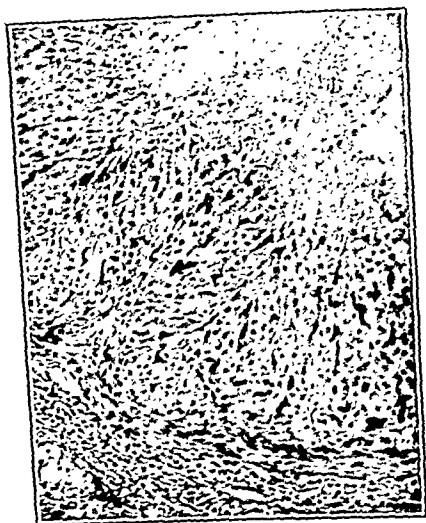


Fig. 7.

Fig. 6.—Photomicrograph. High power magnification of rabbit ovary. Luteinizing follicle showing proliferation of granulosa cells and of theca.

Fig. 7.—Photomicrograph. High power. Rabbit ovary. Corpus luteum of forty-eight hours' duration showing proliferation of capillaries.

*C. Urine for Injection.*—In order to avoid doubtful and false negative results, the urine to be tested must be a satisfactory specimen. The patient is instructed to restrict fluids the evening before the specimen to be tested is collected, to void before retiring and to promptly bring the first voided morning specimen to the laboratory. The urine should be of a specific gravity of 1.015 or more. The amount of urine injected intravenously by different workers has apparently varied more than any other single detail of the test. Friedman<sup>7</sup> first recommended a single dose of 5 c.c. Later<sup>7</sup> he recommended the use of 24 c.c. in six doses of 4 c.c. each. Other workers have used single doses of 10 c.c., 2 doses of 10 c.c. each at twenty-four-hour intervals, and still others have injected concentrates of the hormone obtained by alcohol precipitation. The tests in the first half of our series were done with a single injection of from 10 to 15 c.c. At present we regard 30 c.c. in two doses of 15 c.c. each as more advisable. The

doses are given four hours apart in order to avoid injury to the rabbits by a large amount of urine which is always somewhat toxic.

The use of a large amount of urine does not lead to false positive results, since as much as 100 c.c. of urine from nonpregnant women have been injected in divided doses and the equivalent of 200 c.c. of male urine concentrated by alcohol precipitation have been injected in a single dose without producing false positive results.

In a certain number of cases (3 per cent in this series), the urine on injection proves toxic and results in the death of the animal. The urines in this series which proved toxic were either turbid with bacteria or were obtained from patients who were receiving medication. The urine may be purified before injection into another rabbit by the method recommended by Zondek as follows: The hormone is precipitated by adding five volumes of 95 per cent alcohol to 60



Fig. 8.

Fig. 8.—Photomicrograph. Low power. Rabbit ovary. Positive test of seventy-two hours' duration (corpus luteum).



Fig. 9.

Fig. 9.—Photomicrograph. Low power. Rabbit ovary. Positive test showing follicles of eight days' and of twenty-four hours' duration. (Corpus luteum, corpus hemorrhagicum.)

c.c. of urine. The precipitate is collected by centrifuging in large tubes, and the supernatant fluid is discarded. The precipitate containing any anterior pituitary hormone present is dissolved in as small an amount of distilled water as possible and extracted by shaking for fifteen minutes with three volumes of ether to remove the toxic substances. Folliculin is also removed by the ether extraction. The ether washings are discarded and the excess ether removed from the solution by aeration. The solution is then made up to about 10 c.c. with distilled water and injected slowly in a single dose. The 60 c.c. volume is chosen to allow for loss of about one-half of the hormone in handling.

*D. Examination of Ovaries.*—While in general one hundred hours is the time interval allowed when using the immature mouse as the test animal, Zondek<sup>11</sup> has reported a method of shortening the time of the mouse test from one hundred

to seventy-two hours. The modification is as follows: A 30 c.c. sample of urine is extracted with three to four volumes of ether. The ether washings are discarded and 0.9 gm. of glucose is added to the urine residue. The usual injections are then given.

In the test using the rabbit, Friedman<sup>2</sup> recommends forty-eight hours, and this is the time interval which has been generally adopted. Normally after copulation, twelve hours are required for the development of corpora hemorrhagica in the rabbit's ovary.

In a negative test the ovaries are white, and while numerous ripe follicles may be seen, there are no areas of hemorrhage present. In a positive test at forty-eight hours the ovaries are large, and show a number of hemorrhagic follicles which are easily recognizable grossly. Microscopically not only is hemorrhage in the follicles striking but there are also a number of apparently recently proliferated capillaries and the presence of one or more layers of maturing lutein cells.

The gross and microscopic examination of the ovaries (see Figs. 1 to 9 incl.) removed at intervals of from one to thirteen days after injection of urine from a

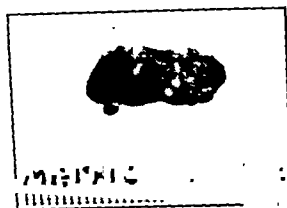


Fig. 10.—Gross photograph. Natural size. Rabbit ovary. Subinvolution of ovary (twenty-eight days after injection of urine of pregnant woman).

pregnant woman show important changes in the development of corpora lutea from the ripe follicles. At the end of twenty-four hours some of the follicles show a small central area of hemorrhage and lutein cells one or more layers in thickness apparently developing from the granulosa cells and from those of the theca interna. There is also visible in some of the follicles evidence of proliferation of new capillaries. It is probable that the hemorrhage in the corpus hemorrhagicum is due not to rupture of the follicle at ovulation but to bleeding from capillaries proliferating to maintain the developing corpus luteum. This view is supported by the fact pointed out by Friedman<sup>2</sup> that corpora hemorrhagica and corpora lutea may be formed whether or not ovulation occurs. In addition to maintaining the corpus luteum newly developed capillaries share in the organization of the follicular hemorrhage. At the end of forty-eight hours some of the follicles show larger areas of hemorrhage and a thickened collar of lutein cells, and quite a number of others show changes characteristic of the twenty-four-hour reaction. At the end of seventy-two hours the follicles show, in addition to more lutein tissue, well-developed evidence of organization of the central hemorrhage. By the fifth day the process is quite distinctive grossly, and at the end of a week the corpora lutea are prominent and unmistakable. Ovaries at five- and

eight-day intervals show follicles which grossly and microscopically can be dated as fully developed one-, two-, three-, five-, and eight-day reactions, indicating that the stimulus of the hormone is exerted continuously for about eight days.

Sections stained with Scharlach R. show a scanty deposit of lipoid globules in the lutein cells as early as twenty-four hours and increasing amounts of lipoid in the older lutein cells. Those of three and five days show abundant globules.

The majority of workers have accepted gross inspection of the ovaries as sufficiently characteristic for a final diagnosis in the test for pregnancy. While in the vast majority of instances we have found it satisfactory, our one false positive reaction would have been avoided by microscopic examination. This animal was injected with urine from a case of extensive tuberculous salpingitis and oophoritis, and forty-eight hours later showed hemorrhagic follicles. The test was repeated in two separate rabbits, both of which showed similar hemorrhagic follicles in the ovary which were grossly indistinguishable from corpora hemorrhagica. Microscopic examination, however, showed that there were degenerative and necrotic changes in the wall with hemorrhage into the follicles but no evidence of lutein tissue formation.

After surgical removal of the affected ovary and fallopian tubes of the patient, the Friedman test was negative on repeated trials.

These findings were further controlled by the injection of urine from five cases of advanced pelvic tuberculosis which we obtained from the Cleveland City Hospital through the courtesy of Dr. S. E. Wolpaw. All of these rabbits gave negative results. In 42 tests subsequent to this error all positive tests were controlled by removing one ovary at laparotomy and examining it microscopically for lutein tissue after frozen section. These operated animals may be used for a second Friedman test in about four weeks.

The one false negative test was obtained in the first half of this series when a single dose of 15 c.c. of urine was used. The specimen of urine had a specific gravity of 1.014. On repeating the test three weeks later, using the same rabbit and 15 c.c. of urine with a specific gravity of 1.028, a positive result was obtained.

Some workers have retested the animals giving negative results in cases for diagnosis with known positive urine. In the light of the variable reaction capacity of the animals this method of control is certainly indicated especially when less than 30 c.c. of urine is injected. In the latter part of our series 29 negative tests were controlled by injection of positive urine and this control of negative tests is now a routine procedure.

Since freshly voided urine from a known case of pregnancy is not always available for injection, the majority of the controls on negative tests in our series were done with concentrated urine. The concentration may be carried out as described above for detoxifying urine. For concentrating large amounts of urine the original procedure has been modified in order to reduce the amount of reagents required. The method is as follows: 500 c.c. of 95 per cent alcohol are added to 1,000 c.c. of pregnancy urine in a shallow pan and the mixture evaporated to dryness by fanning (in a hood). The sediment is taken up in

200 c.c. of 60 per cent alcohol and shaken vigorously in a shaking machine for one-half hour. The material is then filtered, the residue discarded and the hormone is precipitated from the filtrate by the addition of five volumes of 95 per cent alcohol and allowed to stand in the ice box overnight for complete precipitation. The supernatant fluid is discarded and the precipitate is dissolved in as small an amount of distilled water as possible (about 50 c.c.). This solution is extracted with three volumes of ether in order to remove the folliculin. The ether washings are discarded and the solution is aerated to remove the excess ether. An amount of the material equivalent to 40 c.c. of the original specimen is distributed into small tubes, each tube to be used as a positive control for negative tests. This measured concentrate is again precipitated with five volumes of alcohol and stored under alcohol in the ice box ready for use. (The amount equivalent to 40 c.c. of urine was selected in order to allow for some loss of the active principle in handling.) Before a lot of the concentrate is used for controlling negative tests a sample of it is injected into a suitable rabbit to determine its potency. Before the injection into a rabbit the alcohol is poured off and the sediment is taken up in a few cubic centimeters of physiologic saline solution.

#### RECOMMENDED PROCEDURES FOR THE FRIEDMAN TEST

1. Selection of ordinary mature domestic female rabbits weighing from 2 to 4 kg.
2. Isolation of the animals in individual cages.
3. Use of morphine sulphate intravenously as an anesthetic.
4. Preliminary laparotomy and inspection of ovaries with selection of animals with ripe or nearly ripe follicles but no corpora hemorrhagica or corpora lutea.
5. Injection intravenously of 15 c.c. of clear, filtered, first morning specimen of urine, warmed to body temperature, of a specific gravity of 1.015 or higher. This dose is repeated in about four hours.
6. Forty-eight hours after the first injection the wound is opened and the ovaries are reinspected. If the ovaries grossly appear positive, one ovary is removed and examined microscopically by frozen section. The presence of lutein tissue in the wall of one or more follicles associated with more or less hemorrhage into the follicle constitutes an undoubtedly positive reaction.
7. If the ovaries show no corpora hemorrhagica the wound is closed and the rabbit injected with 15 c.c. of known positive urine or concentrated urine as described above. A tentative report of a negative test is given. Forty-eight hours after the injection of the positive control urine the wound is opened and the ovaries are reinspected. If the ovaries now show corpora hemorrhagica and lutein tissue is observed microscopically, the final report of a negative test with the urine for diagnosis is given.

If facilities are not available for making frozen sections, a reinspection of the ovaries on the fifth or sixth day after injection will show sufficient lutein tissue grossly to make an undoubtedly positive diagnosis possible.

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## AN IMPROVED ELECTRODE FOR THE MEASUREMENT OF POTENTIALS ON THE HUMAN BODY\*

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IN AN attempt to map the electrical potentials of the various parts of the body in connection with studies of the relationship of such potential differences to metabolism, it was necessary to use a large number of relatively non-polarizable electrodes. Such an electrode has been described by Alvarez, Freedlander, and Clark<sup>1</sup> and used by Purdy and Sheard<sup>2</sup> in connection with studies of metabolism. Our experience with the Alvarez electrode led us to attempt to eliminate the difficulties which we experienced: clumsiness due to the comparatively large size of the "H" tube; frequent cracking of the cross member of the tube, especially when this electrode is used with noncooperative psychopathic patients; the variation of conductivity due to changes in the amount of moisture in the gauze wicks; and the change of resistance and potential arising from the loosening of the copper wire embedded in stiff copper amalgam.

Our improved electrode, which is drawn to scale in Fig. 1, consists essentially of an outer tube of one inch diameter, which functions in the same manner as the wick-bearing arm of the Alvarez "H" tube, and an inner tube  $\frac{1}{4}$  inch in diameter, which functions essentially in the manner of the second side of the "H" tube. The chemical composition of the electrode is the same as that used by Alvarez and his coworkers, i.e., saturated lead chloride, lead-mercury amalgam (3 per cent to 97 per cent) and copper wire connection. We, however, have omitted the stiff copper amalgam. The small inner tube is bent

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as shown, and a bulb  $\frac{1}{2}$  inch in diameter blown in the middle of the shorter arm, in order to give a larger cross-section to the amalgam lead-chloride junction. This design prevents spilling of the mercury in case the electrode is tipped. A graduation, etched on the equator of this bulb, serves to standardize the level of the mercury-lead amalgam.

This inner tube is mounted by thrusting it through one of the holes of a two-hole rubber stopper. A short tube (B), placed in the second hole, provides for the filling of the electrode and also serves as an anchor for the copper wire leading off from the electrode (C). This wire is run directly into the mercury-lead amalgam, thus eliminating the stiff copper amalgam used by Alvarez and his coworkers. The copper amalgam is inconvenient in assembly, tends to jar loose with use, and is unnecessary to the proper operation of the electrode. A semi-permeable membrane ("fish-skin" condom, washed in alcohol and ether)

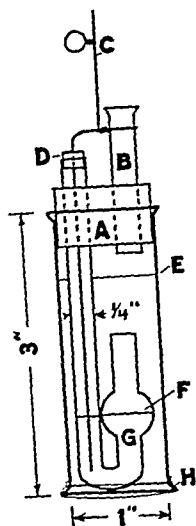


Fig. 1.—Improved electrode. A, rubber stopper; B, open glass tube; C, copper lead-off wire loop for hanging electrode; D, sealing wax plug; E, level of lead chloride solution; F, etched graduation on inner glass tube; G, lead mercury amalgam in inner tube; H, semi-permeable membrane attached by rubber bands over end of 1-inch tube. The lip of the 1-inch tube allows the electrode to be held loosely in clamp, if desired.

stretched somewhat loosely over the bottom lip of the one-inch tube, furnishes a fairly constant moist conductor, and the slight bulge of the membrane under the weight of the solution in the outer tube gives a means of standardizing the pressure of the electrode on the patient's skin. Our experience indicates that electrode pressure on the skin may affect the potentials which are being measured.

A loop in the lead-off wire (C) furnishes a convenient means of hanging a fairly large number of electrodes inside a moist chamber to prevent drying of the membrane and evaporation from the electrode between daily experimental periods.

In actual practice, a single thickness of chamois saturated with lead chloride solution was placed between the electrode and the skin of the patient. It was found convenient to clamp the electrode loosely in a vertical position (with

TABLE I  
VARIATION OF POTENTIAL DIFFERENCES DEVELOPED BY THE IMPROVED ELECTRODES

|                                          | TIME<br>ELECTRODES<br>IN USE<br>(MIN.) | ELECTRODE NUMBER |      |      |      |      |      |      |      |      |      | AVERAGE<br>MV.<br>VARIATION | AVERAGE<br>VARIATION<br>PER MIN. |
|------------------------------------------|----------------------------------------|------------------|------|------|------|------|------|------|------|------|------|-----------------------------|----------------------------------|
|                                          |                                        | 1                | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |                             |                                  |
| <i>Potential Variation in Millirolts</i> |                                        |                  |      |      |      |      |      |      |      |      |      |                             |                                  |
| Highest variation                        | March 6                                | 0.60             | 0.60 | 0.55 | 0.50 | 0.50 | 0.40 | 0.30 | 0.10 | 0.35 | 0.15 | 0.405                       | 0.0068                           |
| Freshly prepared electrodes              | March 7                                | 0.00             | 0.10 | 0.00 | 0.05 | 0.00 | 0.05 | 0.10 | 0.10 | 0.05 | 0.20 | 0.065                       | 0.0013                           |
| Four days later                          | March 10                               | 0.20             | 0.15 | 0.20 | 0.00 | 0.05 | 0.10 | 0.40 | 0.10 | 0.20 | 0.15 | 0.155                       | 0.0041                           |



patient prone), thus utilizing the weight of electrode rim and of the liquid on the membrane (*II*) as a standard pressure against the skin. The variation of potential differences on three different occasions between each of 10 different electrodes and a single reference electrode is shown in Table I.

The figures in the table represent 3 out of 20 such determinations, which were picked to illustrate the range of potential variation observed. The electrodes were in use on each day for from forty minutes to one hour. The first row of figures shows the variation of potential in millivolts for each of 10 electrodes on a given day and represents the greatest change observed. On this day the electrodes had been in use for a week or more. The second row represents change of potential during a similar period on the next day when the electrodes had been freshly assembled and, therefore, represents the operation of the electrodes under the most favorable conditions. The last row represents the variation of potentials after the same electrodes had been assembled for four days.

The next to the last column at the right of the table gives the average potential variation for the 10 electrodes in each case, and the final column, the average potential variation per minute. Both are in terms of millivolts. It is therefore evident that when the electrodes are freshly assembled, we may expect an average change of approximately 0.001 millivolts per minute, whereas after four or more days' use the electrodes are likely to develop a potential variation of 0.004 millivolts per minute or more. It is therefore recommended that for accurate work with body potentials, the electrodes be freshly assembled every four or five days or whenever a check of potential variation over a period of time shows sufficient change to affect the magnitude of measurements being made.

The figures of Table I were obtained when the electrodes were applied to the human body and under ordinary clinical working conditions, so that temperature of the electrodes undoubtedly varied due to transmission of heat from the body. The variation obtained is therefore not surprisingly large.

#### SUMMARY

A modification of the Alvarez, Freedlander, and Clark electrodes for use in measuring the potentials of the human body has been developed, as shown in Fig. 1. It possesses advantages over the previous electrode of the same general type: handy size, allowing the use of a large number of electrodes; practically the same nonpolarizing qualities as the Alvarez electrode; uniform pressure on the skin; ease of hanging in solution between periods of use; and comparative ease of assembly, which favors the use of electrodes which are in good condition.

In general we feel that our electrode is much more convenient for clinical use, and especially when used for measurements on psychopathic patients.

For the work of assembling and testing out the electrodes we are indebted to Mr. Joseph Barmack.

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## A SIMPLIFIED PSYCHODOMETER\*

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THE measurement of reaction time in fractions of a second following drug action in man has offered difficulties to investigators generally. The stop watch is costly and fragile in the hands of students. The split second timer described by Guthrie<sup>1</sup> in 1932, is not readily applicable to visual-mental-muscular reaction time tests. Dr. Metfessel's psychodometer is an excellent reaction timer for such psychophysiologic tests, but it is both complicated and expensive.

To obtain a less expensive, more rugged, and equally satisfactory apparatus for many types of experiments, the accompanying simplified modification of Dr. Metfessel's psychodometer was devised in connection with a study of the effect of caffeine upon the rapidity of mental and physical coordination and reaction in man.

The relatively simple psychodometer is illustrated in Fig. 1. It involves primarily the use of an impulse counter which records time intervals in one-hundred-twentieths ( $\frac{1}{200}$ ) of a second. The other parts can be assembled usually from miscellaneous equipment around any physical or physiological laboratory with very little expenditure.

The impulses were furnished by stepping down the 60-cycle alternating current of 110 volts to about 6 volts. Fig. 1 shows the apparatus to be quite readily portable and durable. Both of these factors are highly desirable in laboratory use. In Fig. 2, the schematic diagram of the psychodometer, check lights have been omitted in order to simplify the circuit. It was found that an assistant to the experimenter can easily check the accuracy of the subject's responses in performing the proper act in accordance with the stimulus given. For example, the tapping of the key corresponding to the color of the light stimulus flashed was required in most of the experiments. Auditory or other stimuli could be substituted for light.

The operation of the apparatus is very easy. It requires many more words and much more space to write or tell it than to do it. As the wiring shows in Fig. 2, the closing of a signal switch, for example, "S<sub>2</sub>," determines which of the

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possible lights will be flashed when the circuit is closed subsequently by the operator when he throws the double-pole, single-throw switch, " $S_1$ ." This latter switch also starts the impulse counter instantaneously with the flash of the light (red in this instance). Upon receiving the visual stimulus, the subject closes the proper key ( $K_2$ , in this instance) which stops the impulse counter, but does not put out the light until the operator opens the double-pole switch, " $S_1$ ." *Note:* As a matter of fact, in this simplified apparatus without the check light circuit, the tapping of any one of the keys  $K_1$ ,  $K_2$ , or  $K_3$ , stops the counter. The subject, however, is not aware of this fact and the operator's

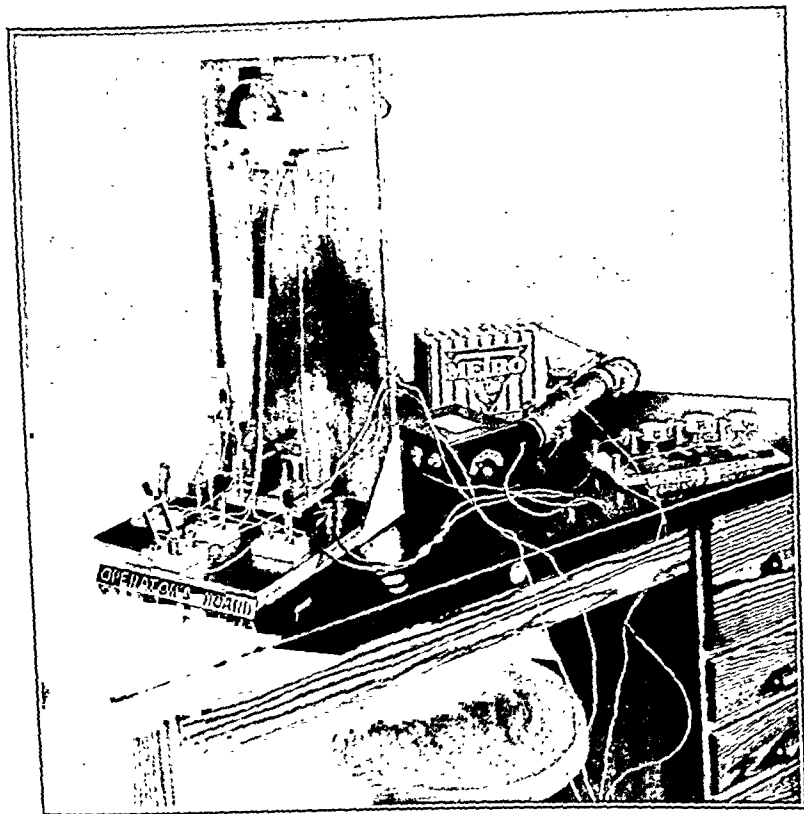


Fig. 1.—Simplified psychodometer for human experimentation.

assistant observes whether or not the subject utilizes the correct key in accordance with the light flashed.

The operator is obscured behind his apparatus board (any large piece of wall-board serves effectively) in order that the subject cannot observe any of the operator's movements. The subject is seated at the opposite end of a 12-foot laboratory table with the subject's tap keys available. Wires of variable length may be used here to allow any desirable distance to intervene between subject and operator. The tap-keys are provided with large wooden discs so that the finger will not readily slip off, as the subject is required to hold the key down temporarily until the operator puts out the light by opening switch, " $S_1$ ." The operator's recording assistant is seated at a table near the subject

and in full view of the tap-keys. He notes any errors in the tap-key choice which the subject may make. The subject sits with his right hand on the table in front of the tap-keys and with his left hand resting upon his left knee. In this way, all of his movements to the tap-keys are as nearly equidistant as possible.

### MANIPULATION

The operator reads the stationary reading of the impulse counter and gives the dial number aloud, which is recorded by his assistant as the original reading for that trial. At variable time intervals after giving an original reading in each case, a light is flashed and the counter starts simultaneously. The subject observes the light flash stimulus, determines its color, and stops the counter by

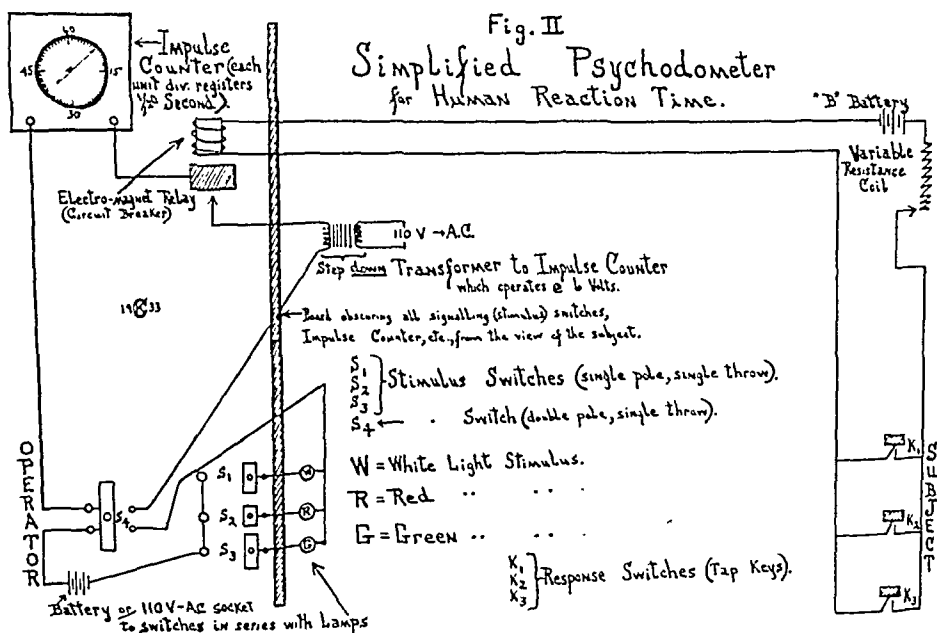


Fig. 2.—Schematic representation of simplified psychodometer for studies in human reaction times.

closing the proper tap-key. The operator then reads the dial number as the final reading for that trial. The difference between the two readings is obviously the total reaction time for this eye-hand mental decision, and muscular response-action phenomenon. Twenty-five trials or more may be averaged for greater accuracy.

The comparisons of reaction time under the influence of various drugs are compared readily, and their effect upon complicated psychophysiologic reaction times recorded quantitatively.

The valuable assistance of Professor Clarence J. Pietsenpol of the Physics Department and his assistant, Mr. Ralph L. Hirschel, is gratefully acknowledged.

### REFERENCE

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## A MODIFICATION OF HILL'S RADIOPAQUE MASS FOR THE INJECTION OF LUMINA<sup>2</sup>

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A RELIABLE technic for the injection of radiopaque materials into the lumina finds many applications in anatomical, pathologic, and surgical studies of lymphatic, vascular, and duct systems. Probably the most satisfactory material for the injection of dead subjects is the bismuth oxychloride mass of Hill<sup>1</sup> when the suspension is properly made. Many workers, however, are unable to obtain consistently good results using the Hill preparation be-

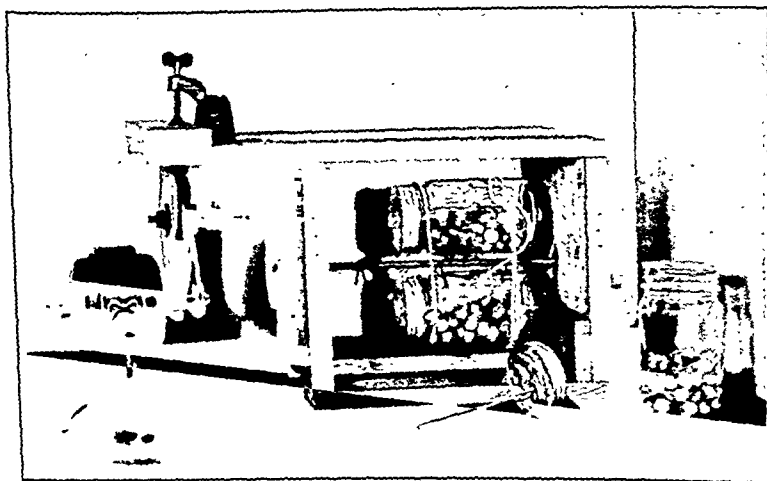


Fig. 1.—An improvised jar or ball mill showing two fruit jars mounted in place and containing marbles. Speed, 10 r.p.m. A jar with a top adapted for injection is shown.

cause they cannot or will not follow the rather troublesome method of preparing the mass. A simple, easily duplicated method for the preparation of a uniform product has been evolved and will be described.

### PREPARATION OF THE SUSPENSION

The proportion of acacia to bismuth oxychloride taken is as one is to two. The quantity of water varies with the density of suspension desired. The unmixed ingredients are placed in a glass fruit jar or ball mill crock one-third filled with ordinary glass marbles. This is placed on a ball mill (Fig. 1), such as is commonly used by chemists for grinding materials extremely fine, and is allowed to grind for two to three days. These suspensions are still unstable and will settle out appreciably after standing several days, and

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so it is advisable to regrind for an hour or two before making injections. Table I illustrates certain suspensions and designates their use.

TABLE I

| ACACIA<br>GM. | BiOCl<br>GM. | WATER<br>C.C. | TOTAL VOL.<br>IN C.C. | PERCENTAGE<br>BiOCl | GM. BiOCl<br>PER C.C. | SUITABLE FOR<br>INJECTION OF: |
|---------------|--------------|---------------|-----------------------|---------------------|-----------------------|-------------------------------|
| 50            | 100          | 450           | 500                   | 16.5                | 0.2                   | Lymphatics                    |
| 110           | 220          | 500           | 600                   | 26.5                | 0.37                  | Arteries and<br>capillaries   |
| 165           | 330          | 500           | 650                   | 33.2                | 0.51                  | Arteries                      |

## INJECTION TECHNIC

a. *Lymphatics*.—Lymphatic injections are usually made with a Luer syringe fitted with a 27 gauge needle.

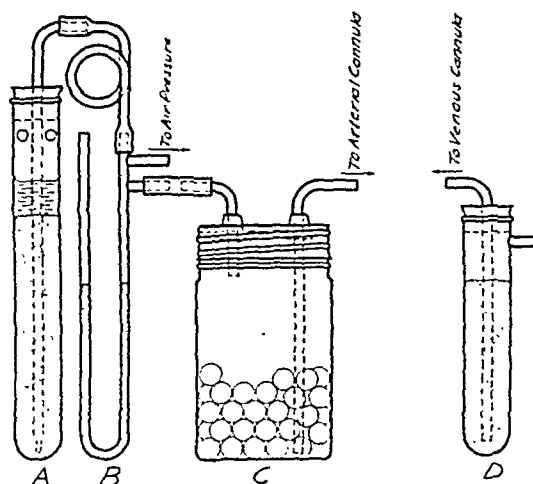


Fig. 2.—Injection system. A, Pressure regulator containing mercury topped with water to eliminate mercury spray. The inner tube can be raised or lowered so as to give the desired injection pressure. B, Mercury manometer. C, Fruit jar used in the grinding on ball mill shown with a top adapted for injection. D, Pressure regulating overflow trap containing mercury. The inner tube is adjustable to give the desired pressure in the venous tree. To be used only when visualization of the venous bed is required.

b. *Arteries*.—Arterial injections are best made by cannulating the artery and injecting under constant pressure using the system illustrated in Fig. 2, A, B, and C. The injection pressure is important and will vary with the density of the suspension, the end-result desired, and the species of animal injected. The small arterioles and capillaries are easily ruptured when a low density mass is injected under high pressure. The proper pressure for injection is readily determined and is best done by beginning the injection at a low pressure and then increasing the pressure in steps of 10 mm. of mercury, taking roentgenograms at each level of pressure. The injection is usually complete within five minutes. The interval of pressure throughout which satisfactory pictures are obtainable is sufficient that once it is determined for a species, it will hold for all individuals of this species (Figs. 3 and 4). Likewise, this is a good procedure to follow when injecting a valuable specimen.

c. *Veins*.—Simultaneous injection of arteries and veins produce confusing results because of the inability to differentiate between the arterial and venous systems. However, if the initial injection is made with a low density mass at a low pressure, a satisfactory outline of the arteries is obtained. Then, by increasing the pressure of injection and retarding the escape of the excess mass from the venous side by causing it to flow through a pressure regulating trap, Fig. 2, *D*, a roentgenogram of both arterial and venous beds may be

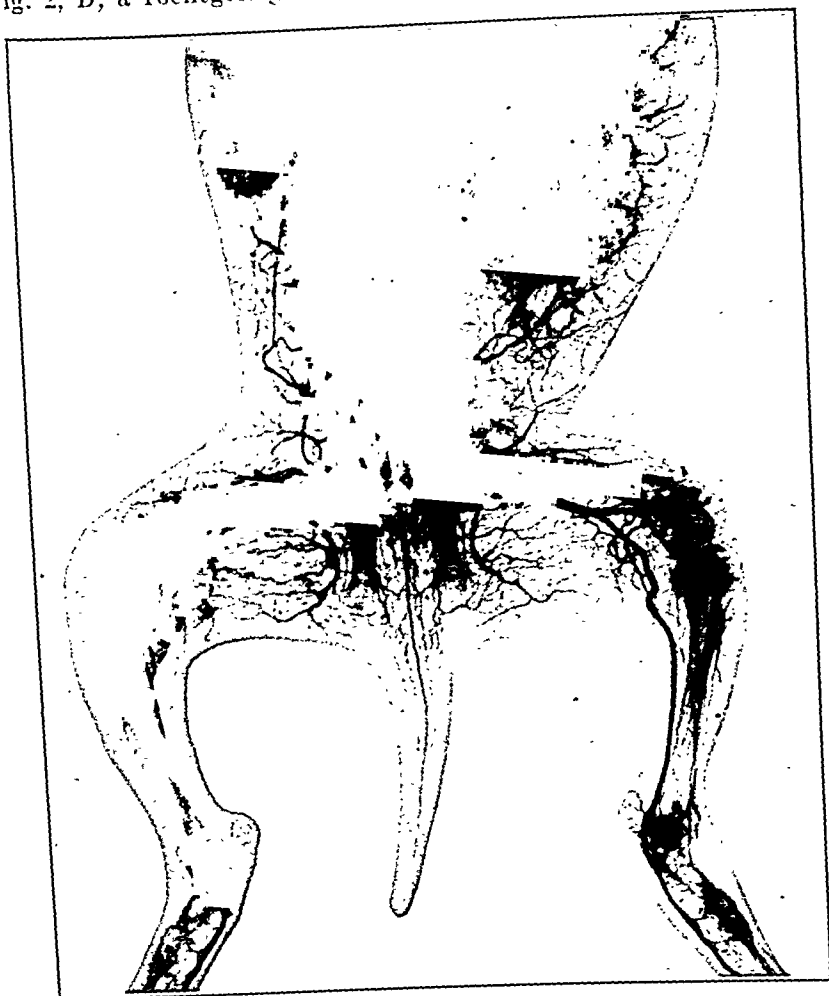


Fig. 3.—A photographic reproduction of a blocked-out roentgenogram of a rabbit injected at 120 mm. Hg pressure with a mass containing 0.5 gm. of BiOCl per c.c. No retouching has been done. Same animal as is shown in Fig. 4. The smaller vessels are lost in the reduction.

secured. Finally, a suspension of starch in acacia solution is substituted for the bismuth oxychloride mass, and the opaque material in the arterial tree is displaced. Since the starch suspension will not pass through the capillaries, a high pressure, 150 mm. Hg. is used in this injection. This will force the opaque mass out of the arteries and permit visualization of the venous bed without the interference of arterial shadows. All roentgenograms should be made while the injection pressure is maintained.

The best results are obtained when the injections are made immediately after death and before the blood has coagulated. If the blood has coagulated, the injection can be done after the clot has retracted and the serum separated.

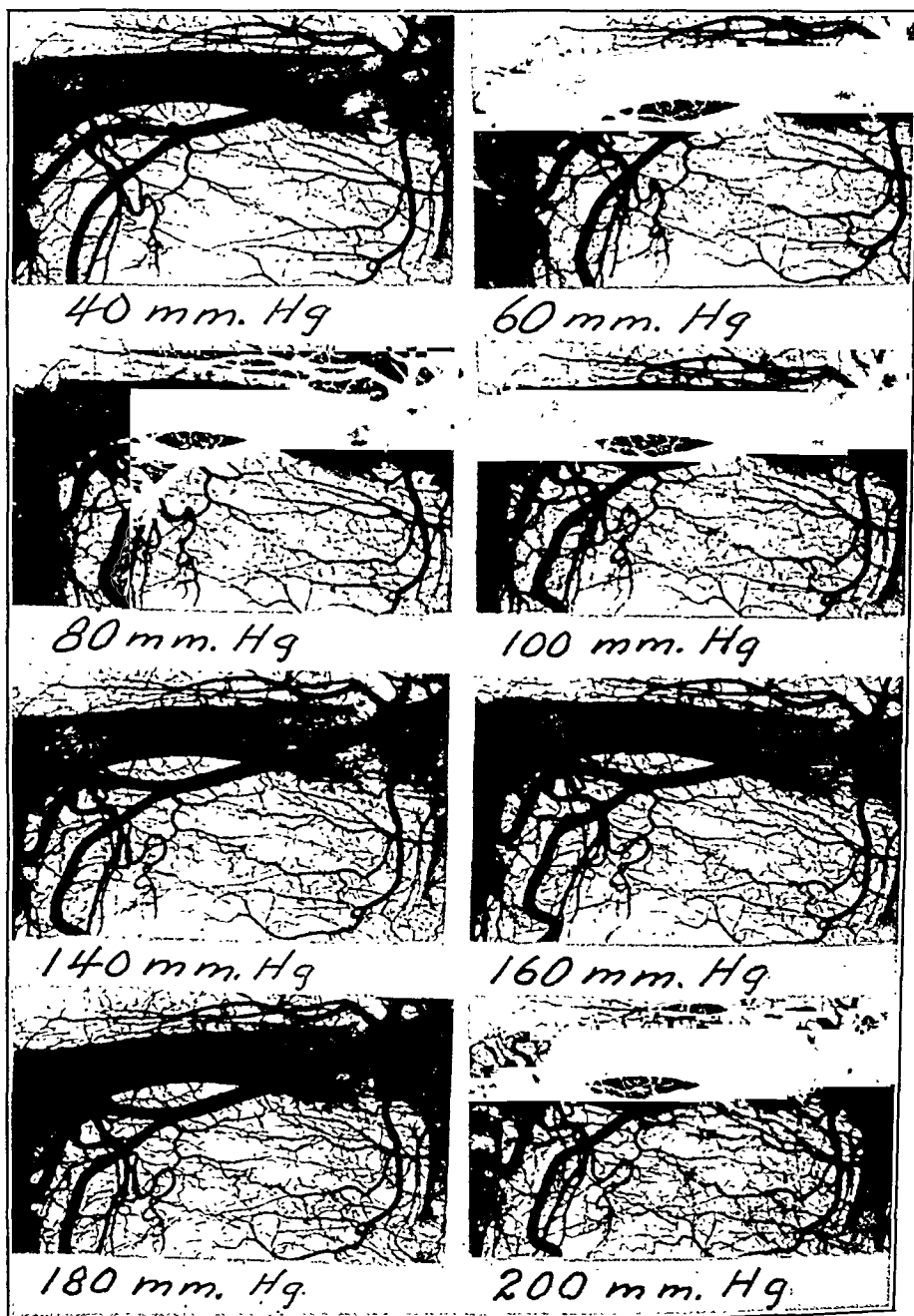


Fig. 4.—Right thigh of animal shown in Fig. 3. Injection pressure as indicated under each successive cut. These are contact prints from roentgenograms which have not been retouched. No vessels show rupture until a pressure of 200 mm. Hg is used; BiOCl extravasated into femur. Stereoscopic roentgenograms clearly show that the nutrient vessels of bones are visualized when injected at a pressure equal to the normal blood pressure of the animal.



With a little experience the proper density of suspension and the correct pressure for injection are readily selected for the problem at hand.

# DISCUSSION

Bismuth oxychloride makes a very satisfactory substance for injection, not only because of the high atomic number of bismuth and its consequent opacity to x-radiation, but, also, because of the physical properties of the compound. The bismuth oxychloride<sup>\*</sup> used in this work is extremely finely ground and will assume very definite physical properties when suspended and ground in acacia solutions. After grinding such a suspension for some hours in a jar mill the particles arrange themselves in chains less than  $\frac{1}{2}$  micron thick and from 3 to 4 microns long. Upon standing for fifteen minutes the chains arrange themselves into spherical rosettes about eight microns in diameter. No larger particles are formed regardless of the density of the suspension, except on long standing.

The greatest density of any mass suggested by Hill contains 0.4 gm. of BiOCl per c.c. and, as he states, this is a paste. By the method of preparation presented here, the most satisfactory mass for arterial injection contains 0.5 gm. per c.c. and has the consistency of a thin cream. Injection of this material at a pressure equal to the normal blood pressure of the animal usually gives satisfactory visualization of the arterial tree extending out to minute arterioles without the fogging attendant upon capillary shadows.

With a minimum expenditure of effort, exacting no individual skill, the mass described can be prepared, and it will have uniform and reproducible composition and physical properties.

# SUMMARY

1. A simple, easily duplicated method for the preparation of a uniform, radiopaque mass, which insures complete injection of lumina, is presented.
2. An injection technic is described.
3. Methods for obtaining visualization of both arterial and venous beds are outlined.

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\*Merek's C. P. Bismuth Oxychloride, which conforms to "Standards of Murray." See *Standards and Tests for Reagents and C. P. Chemicals*, New York, 1927. D. Van Nostrand Co.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**THYMIC HYPERPLASIA:** Follow-Up Study of 30 Cases, Wadlbott, G. L., and Anthony, G. E. *Am. J. Dis. Child.* 47: 34, 1934.

Of a group of 102 cases in which the diagnosis of status thymicolymphaticus was made by various pathologists, 34 were selected in which no adequate cause of death had been determined. Sixty-eight cases were excluded since other conditions, such as hyperthyroidism, birth trauma, gastroenteritis, and respiratory infections, were associated with lymphoid hyperplasia, and in themselves were sufficient to cause death.

In 11 of the 34 cases death occurred without previous illness, and there were no pathologic lesions to indicate the presence of a previous illness. In 16, minor, usually nonfatal, incidents preceded death. In 7, a syndrome developed manifesting dyspnea, stridor, fever, and shock.

The lungs of all these patients presented uniform changes, characterized by capillary congestion, extravasation of blood cells, and edematous fluid. These lesions alternated with areas of emphysema and atelectasis. In 17 cases petechial hemorrhages were present, involving the heart, pleura, lymph glands, and various other viscera. In some, there were dilatation of the right side of the heart, degenerative changes in the liver, and edema and capillary congestion in other organs than the lungs. Hypoplasia of the suprarenal glands and hyperplasia of the lymphoid organs, as noted by other authors, were present.

Comparison of this pathologic process with that reported in anaphylactic shock in man reveals a close resemblance, if not a complete identity, of the two conditions. Eosinophilia of the tissues and an allergic family or personal history point further to this conception.

On the basis of these findings, it is believed that death may be the result of a primary anaphylactic edema of the lungs and ensuing asphyxiation. Such a theory can be upheld only if one assumes: (1) that anaphylactic shock may occur from incorporation into the body of nonprotein substances; and (2) that absorption of shock-producing antigen may take place by ways other than by injections. Evidence is presented, both from personal experience and that of others, which tends to confirm these facts.

**BLOOD LIPIDS, in Children With Scarlet Fever and Rheumatic Disease,** Kaiser, A. D., and Gray, M. S. *Am. J. Dis. Child.* 47: 9, 1934.

Values for blood lipids in 29 normal children between the ages of five and sixteen were fairly constant, but were considerably below the accepted averages for normal adults.

Values for blood lipids in 43 children convalescing from scarlet fever were practically the same as in normal children. Deviations from the mean were considerably greater, especially for cholesterol.

Similar values for lipids were found in 22 children with acute rheumatism and in 20 with chronic rheumatism. The standard deviations were much greater for the children with rheumatism than for the normal children.

Values for blood lipids deviated considerably from the normal in nephrosis and in nephritis.

Values for blood lipids were not influenced by ordinary variations in diet. The deviations from the mean noted in normal and in sick children seemed to bear no relationship to an increased or diminished intake of fat and of carbohydrates.

No appreciable difference was noted between determinations made on boys and those made on girls. Similar deviations were found in both sexes.

Determinations made in the summer months were uniformly lower than determinations in the winter months.

Studies on the blood lipids of children with rheumatic disease and with scarlet fever revealed values similar to those found in normal children. The standard deviation from the mean was considerably greater in the children with these infections than in the normal children. It is quite likely that the infection, rather than the varied amount of fat ingested, was responsible for the increased deviations.

**SEMEN: A Differential Stain that Advances the Study of Cell Morphology, Cary, W. H., and Hotchkiss, R. S. J. A. M. A. 102: 587, 1934.**

The gross examination includes a notation of the amount, which, when complete, and from men under forty years of age, should exceed 3.5 c.c. Lesser amounts should arouse suspicion of deficiency and premature weakening by vaginal acidity, otherwise negligible. Extended periods of sexual abstinence may impart a slightly yellow tinge to the normal grayish opaqueness. Normal specimens commonly show a slight degree of viscosity, which is easily demonstrated by slowly expelling a drop of semen from a pipet. Absence of viscosity with a lessening of opaqueness points to reduced cell content, although the reverse deduction does not hold.

An alkalinity giving a pH of from 8.1 to 8.4 has been so constant that it seems an unnecessary refinement except in research study.

Two specimens, one thick and one thin, are promptly prepared for microscopic examination. This is done by gently protecting a full drop and a fractional drop with cover glasses, the former permitting a gross impression of the density and motility of the cellular content, the latter allowing a preliminary study of individual cell morphology. In the thicker preparation, semen of relatively high fertility presents a field teeming with spermatozoa, many of which are rapidly propelled by fast-whipping tails. While universal cell activity would constitute the ideal, one usually notes in comparatively vigorous specimens that these fast travelers are colliding with a certain number of inactive (25 per cent average) and a variable number of sluggish and impotent cells, which gradually sink toward the lower strata of the field. Familiarity with this picture under standard, dry, high power magnification constitutes the measure by which motility is judged. It is a practical observation that a motile field, though definitely subnormal, is apt to convey a favorable impression on the inexperienced or occasional examiner, but reverse errors in interpretation are rarely made. In the author's experience the practical aspects of motility are that a large number of highly motile cells are essential to fertility; and when a specimen of normal quantity and rich cellular content shows 25 per cent or more of these dynamic cells, a rating of relative fertility must be assumed unless strongly disproved by other indexes to be described. Such specimens usually meet other major requirements of fecundity.

The following technic permits illustration of the head, midsection, and tail of the sperm.

A. Prepare thin cover slip smears as used in the preparation of blood for staining.

B. Fixation in Schaudinn's solution:

1. Immerse for one minute in 7 per cent solution of corrosive mercuric chloride, 2 parts, and absolute alcohol, 1 part.
2. Immerse for one-half minute in 50 per cent alcohol.
3. Immerse for one-half minute in distilled water, 3 ounces, and tincture of iodine, 2 drops.
4. Wash in tap water.

C. Staining Process:

1. Immerse for one-half minute in aqueous solution of eosin, 5 per cent.
2. Immerse for one minute in 50 per cent alcohol, 3 ounces, and concentrated hydrochloric acid, 2 drops.

3. Wash in distilled water.
  4. Immerse for two and one-half minutes in hematoxylin.
  5. Immerse for one minute in distilled water, 3 ounces, and glacial acetic acid, 2 drops.
  6. Wash in distilled water.
- Dry and mount.

In appraising seminal specimens, deduct the percentage of abnormal forms found from the total cell count. The resultant figure, when correlated with other data, is of great importance in the more accurate evaluation of the semen. For instance, 40 per cent of inactive cells with 25 per cent abnormal forms would indicate more serious impairment and need for greater improvement in a specimen with a count of 60,000,000 or less than would obtain with a cell count of 100,000,000.

**ENTERITIS: Acute, in Infants and Young Children: Bacteriologic Studies, Cooper, M. L., Keller, H. M., and Johnson, B. Am. J. Dis. Child. 47: 388, 1934.**

The bacteriologic methods presented were used in the isolation of streptococci from 65 per cent of 46 infants and children with acute enteritis.

Morphologic and cultural characteristics of these streptococci alone were found to be insufficient for identification.

Marked similarities in the fermentations of carbohydrate were noted in the streptococci isolated from the involved intestinal tracts of patients at necropsy, from the stools of patients, from the throats of patients, and from the intestinal tracts of animals which had received injections.

All the cultures were insoluble in bile with the exception of two, which were questionably so.

For the reasons stated it is suggested that the term *Streptococcus micro-apoikia* (small colony) be employed to designate this group of microorganisms, and that the term *Streptococcus micro-apoikia enteritidis* be employed to designate the strain responsible for the production of enteritis in the patients who were the subjects of this study.

**BLOOD, Studies of in Normal Pregnancy, Dieckmann, W. J., and Wegner, C. R. Arch. Int. Med. 53: 188, 1934.**

Previously reported hemoglobin, hematocrit, and erythrocyte values in pregnancy are, in many instances, at variance with each other, and the results are inconclusive because, with but few exceptions, the determinations were made with methods and apparatus which were not standardized, and they were not made on the same women throughout pregnancy. They are of value only in that they indicate the direction of the change.

Determinations of these constituents and of the blood volume were made on various groups of women for the different periods of pregnancy. While the means show a decrease in each substance, reaching a minimum in the last ten weeks, calculation of the difference between the means demonstrates that the change is of no significance.

Similar studies in which the same women were followed throughout pregnancy and the puerperium indicate that the following changes occur in the blood:

There is a definite decrease in the hemoglobin per hundred cubic centimeters of blood. The maximum decrease, amounting to 15 per cent, is from the twenty-sixth to the thirty-fifth week. At two weeks postpartum the hemoglobin is 17 per cent, and at eight weeks it is 14 per cent, below normal.

There is a slight but definite decrease in the hemoglobin per kilogram during pregnancy, and a more marked one after delivery. Apparently there is an attempt during pregnancy to compensate for the increase in tissue by increasing the hemoglobin, but postpartum the stimulus is gone, and there is a lag in the production of hemoglobin.

The changes in the hematocrit value are similar to those in the hemoglobin content. The maximum decrease during pregnancy is 11 per cent. The recovery after delivery is more rapid, although at eight weeks postpartum the hematocrit value is still below normal.

The changes in the erythrocytes are also similar, but the count is normal at three weeks postpartum, thus demonstrating the ability of the hematopoietic tissue to produce red cells but its inability to stock them with the normal amount of hemoglobin.

The total amount of hemoglobin shows an average increase of 13 per cent, but individual cases show a marked gain during pregnancy and a retention of much of the gain postpartum.

The total cell volume shows an average increase of 20 per cent, which is even greater in many individual cases, and a retention of many of the cells postpartum.

The increase in plasma volume is definitely greater than the increases in cell volume and hemoglobin. Therefore, these constituents show a relative decrease and an absolute increase.

Since the erythrocyte is only a vehicle for transporting hemoglobin and since cell counts in pregnancy are often misleading, more time and care should be devoted to hemoglobin determinations.

In pregnancy a hemoglobin content under 10 gm. per hundred cubic centimeters of blood should be diagnostic of anemia.

The changes of blood volume, cell volume, and hemoglobin are a part of the mechanism by which the body is able to take care of its own increase in tissue and the metabolism of the fetus, with the expenditure of the least amount of work.

**ANEMIA, Etiology and Treatment of in Pregnancy, Strauss, M. B. J. A. M. A. 102: 281, 1934.**

The "physiologic anemia" of pregnancy is only the effect of hydremia.

The hypochromic anemia of pregnancy is due either to a direct dietary deficiency or to a deficiency conditioned by gastric anaecidity, hypoaecidity or associated defects in the presence of the fetal demand for blood-building materials. It may be completely relieved, either during or after pregnancy, by the administration of iron in suitable (usually large) doses.

The macrocytic anemia of pregnancy is generally due to a temporary lack in the gastric juice of a specific intrinsic factor, which has been shown to be absent from the gastric juice of patients with Addisonian pernicious anemia in relapse. The ultimate complete return of this factor after delivery is hypothecated. In other cases the lack of the extrinsic factor (associated with vitamin B) from the diet may produce similar effects. The macrocytic anemia of pregnancy ordinarily may be completely relieved with liver extract although iron is frequently required in addition.

There is some similarity of the etiologic mechanism involved in the hypochromic anemia of pregnancy and idiopathic hypochromic anemia in the absence of pregnancy, and in the macrocytic anemia of pregnancy and Addisonian pernicious anemia.

The development of anemia in pregnancy may best be prevented by supplying the pregnant woman with an adequate intake of blood-building materials.

**SYPHILIS, Transference of Inguinal Glands in Human, Lunsford, C. J. and Day, P. W. J. A. M. A. 102: 448, 1934.**

The results obtained from the intratesticular inoculation of rabbits with human material from 100 cases of syphilis with a 4-plus Wassermann reaction were as follows:

In 8 cases of primary syphilis 100 per cent of the gland transplants caused positive results in live rabbits.

The glands of 24 patients with chronic syphilis who were under treatment caused negative results in rabbits.

In 38 per cent of 29 cases of untreated latent syphilis, the results in rabbits were positive.

For 6 patients with untreated dementia paralytica inoculations into rabbits gave negative results.

The glands of 12 of 38 patients previously treated for chronic syphilis gave positive results. Six of the 12 patients had received intensive treatment, averaging fifty-two injections of neocarsphenamine and forty-six of mercury.

Three patients with acute syphilis who were adequately treated according to present standards showed live *Spirocheta pallida* in their inguinal glands after a lapse from treatment extending from three to five years. The conditions were diagnosed early; the patients were treated intensively and later were pronounced cured on the evidence of completely negative clinical and serologic findings.

It is concluded that:

1. The diagnosis of primary syphilis by means of gland puncture can be made in 100 per cent of cases.
2. It is probable that patients with late syphilis are not a menace to others while undergoing active treatment.
3. A comparison of the 31.5 per cent positive results obtained in previously treated patients with syphilis with the 38 per cent positive results obtained in untreated patients indicates that, from the standpoint of the biologic cure of the patient, inadequate treatment is very little better than none at all.
4. According to our findings, *Spirocheta pallida* is not present in the cerebrospinal fluid.
5. There is a necessity for frequent reexamination of patients with syphilis, even though it appears that they have been adequately treated according to modern standards.
6. The clinical and serologic tests used as criteria of cure in syphilis are inaccurate and must be revised with increasing knowledge of the disease.

**TISSUE, Traumatic Epithelial Cysts of the Skin, Wien, M. S., and Caro, M. R. J. A. M. A. 102: 197, 1934.**

Traumatic epithelial cysts develop as a result of injury to the skin, usually produced by a blunt or tearing instrument. Such cysts occur most often on exposed sites, such as the fingers and palms, and are especially prevalent in those occupations which predispose to injury.

The origin of the cyst is most probably from a bit of epidermis which has been torn from the surface and implanted in the corium. It may also originate from deeper epithelial structures in the absence of surface injury. Here the cyst may form about a foreign body by proliferation of epithelium from the hair follicles or the glandular structures of the skin. Occasionally a foreign body granuloma with cyst formation may simulate an epithelial cyst of traumatic origin.

**LEUKEMIA, Monocytic, Leukemic Reticulo-endotheliosis, Foord, A. G., Parsons, L., and Butt, E. M. J. A. M. A. 101: 1859, 1934.**

Leucemic reticuloendotheliosis (monocytic leucemia) is a distinct condition characterized by the presence of a clinical picture similar to that in other acute leucemic states and the presence of an extraordinary number of monocytes in the blood, usually immature forms. Pathologically, a diffuse enlargement of the lymph nodes, spleen, and liver is found, and in the microscopic examination of the tissues, widespread proliferation of reticuloendothelial cells is found, to a degree as great as or greater than is usually found in the so-called infiltrates of other leucemias.

A report of four cases conforming to the foregoing feature, with autopsies in two and biopsy in one, is given.

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## EDITORIAL

### Have Hospitals Attained a Standard of Mediocrity?

NOT so very many years ago there were numerous hospitals, many of them relatively large institutions, in which the standards of medical practice and the type of care given to patients were notoriously far below what should be and ought to be expected and demanded.

Realizing this situation—and, occasionally, it was very bad, indeed—the American Medical Association and the American College of Surgeons inaugurated a program of hospital standardization whereby a minimum standard of efficiency was formulated to which all hospitals must conform to be “approved.” The teeth in this edict lay in the fact that no unapproved hospital was deemed suitable for the training of resident physicians without whom few hospitals can function.

As a result, there are today very few hospitals which have not made such changes as were necessary to secure approval, which does not, however,

signify that all "approved" hospitals have now reached and maintain a high standard of efficiency.

If there be, as probably there are, those who doubt this statement, their attention may well be directed to the remarks of Dr. H. A. Christian, Professor of Therapy and Practice of Medicine in Harvard University Medical School.

In the course of an address delivered before the Congress of Medical Education, Medical Licensure, and Hospitals in 1931<sup>4</sup> he said:

"Present efforts at hospital standardization have attained a fairly satisfactory level of mediocrity for hospitals. A few, often perfunctory, changes in past practices of a hospital now will secure for it a Class A rating. The very poor hospital has been forced to improve, and this is the only real good that has resulted from all the work of those organizations which have been concerned in the classification and rating of hospitals.

"Many institutions, instead of receiving vigorous stimulation for betterment, are basking in the calm complacency of Class-A-dom. Regulations of the classificationists are cheerfully omitted in the case of the best hospitals in the realization, perhaps the subconscious realization, of the mediocrity of their standards and the probable deterioration that would result in these hospitals from their uniform enforcement.

"If there is to be any official standardization of hospitals new standards should be set. At present far too many hospitals are rated as satisfactory, that is, are Class A hospitals, for such rating to have much value or to be any real stimulus to improvement. We are too much in the condition of a heaven peopled by perfect beings, the angels. Fewer hospitals should be Class A than at present is the case; in other words, there should be fewer angels."

That these comments are not without some basis the impartial observer will not dispute.

It may be true that monthly staff meetings are held, as required, but how often are they inspiring and how often do the reports of services lead to critical debate or constructive criticism? How often, on the contrary, do they degenerate into mutual admiration societies?

Constructive criticism, debate which leads to study or to fruitful thought should never be painful nor reluctantly received for, as Herbert Spencer wrote:

"Mankind in its progress passes through three stages: the unanimity of the ignorant; the disagreement of the inquiring; and the unanimity of the wise."

Hospital records alone, now proudly displayed by all "approved" hospitals, furnish many startling and at times amusing illustrations of perfunctory emphasis upon ridiculous detail and equally perfunctory compliance with the requirements of hospital standardization.

The purpose of the hospital record is obvious and elementary: to supply for future contingencies a clear, succinct recital of events from which a clear reconstruction of the situation can be formed in the mind of the reader. A story so plain, so complete, yet without verbosity, that any one can readily understand why such a diagnosis was formulated, and just why a particular



method of treatment was followed, or a specific operation was necessary. The record need not be voluminous but it should be thorough, clearly phrased, and complete in its data of a particular case.

Let those who have attempted to utilize hospital records in the compilation of statistical data or the preparation of papers testify if the majority of records in the majority of hospitals conform to the requirements listed.

The following editorial from an official bulletin of the Philadelphia County Medical Society offers these comments:

"Hospital records are voluminous and cover everything that can be discovered concerning the patient, but in many instances are shy such minor details as to which arm or leg or eye was operated upon, why the operation was done, how the operation was done, and an exact statement of the degree of restoration to normal on discharge. These details, when omitted, are filled in from the fertile imagination of the medical attendant at large. It is seldom, indeed, that any hospital record cites the indication for a first operation and much rarer that it mentions the indication for a second operation. On the other hand, the emotional variations of the midnight hours are engrossed for posterity with infinite care by the night nurse in charge, and the marital affairs of the blood cells and bacteria set down with mathematical accuracy bearing unanswerable testimony to the fact that the technician, reached at least the eighth grade."

That examples may be found, and readily, to justify these strictures will require no extensive investigation. Few, indeed, are the "histories" which could be used verbatim in a scientific report.

It may well be suggested, with all due respect to the "approving" organizations, that in the inspection of records, it might be advantageous, not merely to tick off the fact that records are made and filed but to ascertain exactly *what kind* of records.

It is quite true that standardized records are practically a *sine qua non* in standardized hospitals, that they are inevitably or generally, therefore, good records is, to speak mildly, open to grave doubt.

Record inspection will invariably present an orderly array of neatly bound sheets which, through their number, their variety, and the stereotyped marginal printed guides, tacitly make claim to the desirable and admirable quality of thoroughness.

And yet one of those responsible for their existence in later years has this to say of them:

"In a medical ward of a class A teaching hospital I recently saw a Jewess, aged forty-five years. Five minutes' conversation brought out the facts that she had always been in reasonably good health until after the death of her husband a year ago; that she then looked hopefully for support from her eldest son; but that about three months ago she gradually experienced the final and crushing conviction that his talents were limited to the selling of newspapers, which yielded a profit of less than a dollar a day. She therefore, in addition to caring for her home and the younger children, took employment in a restaurant, standing eight hours a day washing dishes. Then came backache, sleepless nights of worry, anorexia, loss of 20 pounds, nervousness, utter

exhaustion, and hospitalization. Cursory examination revealed only the ordinary effects of such a life, including possibly some thyroid disturbance.

"Now, I ask of you sticklers for form and order, what do you suppose that woman's folder contained? Five and one-half closely written pages of matter comprised under twenty-eight captions, all neatly underlined with red ink and ruler! Figure out the time that probably took, and then ask yourselves how much time and energy remained to devote to the clinical problem of that woman. We toil through those five and one-half pages in search of useful bits of information. Here and there we find a few, fragmentary and uncorrelated. In the place for 'social condition' is stated that she is a widow; under 'occupation' it is stated that she is a 'housewife'; under 'marital history' that she has four children, but not a word about that fiasco of the eldest son. The paragraph on 'habits' speaks of loss of weight but gives no hint of the possible cause. Breathlessly we work down to the captions 'complaint,' 'onset of present illness,' and 'course of present illness' and find only some sketchy references to pains in the back, palpitation, breathlessness on effort, gas in the stomach, and so on, but never a word of the restaurant or the thoughts in the poor woman's head. Then comes the sacred array of paragraphs on the various systems, with reiteration of shortness of breath under 'cardiorespiratory system,' of stomach gas under 'gastro-intestinal,' etc., etc.

"The writer of this history was evidently painstaking and industrious, and yet what a mess he made of it! There is not the slightest doubt that if, before he ever set foot in the medical school, he had been confronted with this patient and had been asked to write down what he could find out about her condition, he would have done incomparably better. And as a commentary on the teaching of clinical history taking is not that the height of irony? The reason for this enormity is obvious. The writer of the history has been so occupied in constructing and polishing the frame in order to meet the standard specifications that he has been unable to paint the picture; indeed, he has scarcely seen the patient and her experiences at all.

"This case, to be sure, is worse than many of our hospital clinical histories, but it is none the less a good illustration of a valid general criticism of unrestrained standardization; namely, stereotypism, perfunctoriness, mediocrity. And, parenthetically, the criticism is applicable beyond the subject of clinical records. I have heard members of a hospital staff oppose betterments in organization for the reason that the hospital was already accredited by the American College of Surgeons and the college did not require the change."

Criticism is most valuable when constructive and the suggestions of the same author are therefore worthy of interpolation:

"1. That we abandon the effort to amplify records for the purpose of future statistical researches except for specific objectives in institutions where there is a reasonable prospect of continuity and reliability of data to justify the effort.

"2. That teachers and those who presume to examine hospitals for accrediting purposes shift the emphasis from form to content, that they recognize

merit in any form in proportion as it aids in clarifying the individual case, and demerit in proportion as it exhibits aimless wandering, irrelevant matter, redundancy, disjointing of related facts, or otherwise clouds the issue.

"3. That the technic of history taking be regarded as an intellectual rather than a mechanical process. Histories should exhibit uniform clue-mindedness rather than a uniform system of headings and paragraphing. The record of important phenomena, such as weight loss, blood vomiting or edema, should show in the immediate context the effort to assemble the available historical evidence for or against the various possibilities suggested.

"It will be argued that such freedom in composition may be all right for mature clinicians but not for students; that the latter will overlook important data if they are not required to include all the conventional paragraphs. Of course they will; but so are they under the present burdensome regime; and it is better that they miss some of the kernels of grain while actually looking for them than that they miss them through being more occupied by an elaborate sorting of the chaff."

It is probable that more than one hospital pathologist has been impressed with the multitude of careful and varied preoperative studies carried out during the preparation of the fifty cases required for obtaining the coveted F.A.C.S. and likewise somewhat nonplussed to note their abrupt cessation after the initiation ceremonies are a matter of history.

What conclusion is a Resident Physician to form from such a happening: that such studies are valuable and necessary; that they were based upon real indications in fifty consecutive cases which indications were then no longer to be found; or that they are simply "the bunk"?

What value can he attribute to the histories the sole reason for writing which is that the patient is to be discharged, or the "progress notes" compiled when he has gone home?

It is undoubtedly true that Resident Physicians have been to a greater or lesser extent benefited by the compulsory reformation thrust upon hospitals. They have cause to be thankful that the days have passed when all the emphasis was laid upon the duty the interne owed to the hospital and precious little upon what the hospital owed to the interne.

But the millennium is not yet here and one wonders what would accrue if it became the custom for internes to report, when their hospital days were over, to their Dean who advised them in their student days as to internship, their honest opinion as to their experience, their summation as to what they learned and from how great a proportion of the staff, and to what exact degree, as testified to by actual experience, the "approved" hospital justifies its rating.

It is obvious that by far the greater number of those who embark upon the interne year receive their training, not in great medical centers, not in well-equipped and well-manned clinics, not in hospitals with teaching affiliations, but in the average hospital of moderate size. Nor is it always true that those under whom they serve, with whom they are associated in this formative period which often does and always should exert a profound and powerful influence upon the manner in which these young men will later

practice their profession, are necessarily possessed of the teaching instinct or the ability to pass on to others the lessons they themselves have learned in the School of Experience.

If, as a result of his experience, the Resident Physician does not emerge from his hospital year richer in knowledge and enthusiasm, inculcated with the idea of honest, earnest care and thoroughness in the study of his patients, inspired to give to the life work he has chosen the very best that is in him, and thoroughly imbued with the necessity for continual effort to improve his knowledge and ability; and if the lack of these ideals and qualities can be laid at the door of the hospital and its staff, then that hospital has but ill fulfilled its duty toward the interne, has but ill discharged the responsibility which it has assumed toward him, and ill deserved the "approved" status which it doubtless possesses.

And, by the same token, the Dean or the School by whose advice or with whose tacit assent the student seeks and accepts such an appointment, have ill deserved the confidence and belief he has given them.

"Approved" hospitals do not by mere virtue of that fact necessarily become good hospitals nor, and the two are by no means synonymous, efficient centers of postgraduate instruction.

One's travels need not be wide nor his experience with hospitals extensive to grant the truth of this statement.

It is impossible to reach perfection in a single stride; the standardization program, without doubt, was not regarded by its originators as achieving perfection. But there seems reason to wonder if its initial impetus may not have subsided into a gentle stroll along well-defined but narrow pathways; if the early desire for improvements in order to secure approval may not have simmered down to the gentle "complacency of Class-A-dom"; and if its main result is not in some danger of becoming simply a "standard of mediocrity."

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—R. A. K.

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## CLINICAL AND EXPERIMENTAL

### STUDIES IN BACTERIOPHAGE\*

#### I. THE BEHAVIOR OF THE BACTERIOPHAGE AND THE BACTERIA IN THE LESION AFTER THE TREATMENT OF ACUTE STAPHYLOCOCCUS SKIN INFECTIONS WITH BACTERIOPHAGE

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#### INTRODUCTION

IN THIS and in several subsequent papers we shall endeavor to report our laboratory observations in connection with the clinical use of bacteriophage. In reviewing the voluminous literature on the subject of bacteriolysis since the first reports by Twort<sup>1</sup> and d'Herelle<sup>2</sup> one finds that most of the articles fall into two groups: one confines itself to various aspects of the phenomenon in the test tube, while the other concerns itself purely with its clinical application. Only a few authors have reported experiences with the bacteriophage both in the clinic and laboratory and have attempted to set up controlled conditions for their observations. The reports of test tube phenomena have been contributed largely by investigators trying to work out some of the fundamental principles of the reaction, without attempting to apply them clinically. Their work has been of prime importance for our understanding of the nature of the phenomenon and for establishing a rationale for its use, but many of the phenomena which have been observed in vitro cannot be duplicated in vivo, and most of the reports of the clinical application of bacteriophage indicate that the results obtained have been poorly controlled by laboratory observations. The majority of clinical reports have been favorable.

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Failures have seldom been published. One does not persist in a method of treatment which does not have some degree of success. Therefore, only those who have had success have been able to collect a series of cases sufficiently large to be impressive, but it may be seriously questioned whether bacteriophage actually changed the course of some of the self-limited diseases which have been treated with it. The commercial exploitation of bacteriophage has resulted in its wide use by physicians who are not in a position to determine the potency of the products which they employ and who have made no attempt to set up control series sufficiently large to make it possible for them to properly evaluate their results.

We seriously question the wisdom of this uncontrolled use of bacteriophage at the present stage of our knowledge, because its successful use probably depends upon a combination of conditions intrinsic in the patient, in the bacterium concerned, and in the lytic principle available, as well as upon the manner in which these are brought together. Certain minimal essentials would seem to be required for success, a potent bacteriophage, a susceptible infecting organism, an adequate and intimate contact of bacteriophage with infecting organism, and an environment favorable to or at least not antagonistic to the interaction of these two. If the four conditions for success are absent or only partially fulfilled, bacteriolysis, *in vivo*, can hardly be expected to occur. It is not likely to occur with an impotent bacteriophage, with a resistant strain of bacteria, with poor contact of bacteriophage with organism or in an environment unfavorable or antagonistic to the interaction of these two. Whatever benefit may accrue from the injection or administration of the lytic products of bacteria as antigens has been given very little consideration. The whole procedure is still in the experimental stage and unless an effort is made to find out what the necessary conditions for success are and how to set them up, failure will be the rule and the whole subject will be brought generally into the disrepute in which it already finds itself, in the minds of some of the more conservative clinicians and bacteriologists. Extravagant claims for bacteriophage have been made largely by hyperenthusiasts or by those financially interested in its commercial success. This situation is to be deplored, for it seems fairly certain that in the hands of some critical investigators, favorable results have been obtained, results which at times have convinced both the physician and the patient that bacteriophage has some virtue as a method of therapy.

We have been impressed by incidents of this kind and have set out to clarify, if possible, certain obscurities with regard to the behavior of bacteriophage after its injection into the body and the behavior of the bacteria in the lesion after subjecting them to contact with the bacteriophage. In this paper we shall consider the following questions:

1. Does the hemolytic property of certain strains of *Staphylococcus aureus* bear any relationship to their resistance or to susceptibility to bacteriophage?
2. Do the bacteria in the lesion change their cultural characteristics after a single injection of bacteriophage?
3. Do the bacteria become more susceptible or more resistant to the action of bacteriophage?

4. Are the bacteria destroyed in the lesion by the action of bacteriophage?
5. Does the injected bacteriophage persist for any length of time in the lesion?
6. Does the injected bacteriophage increase in potency or amount in the lesion as d'Herelle has claimed?<sup>3</sup>
7. Does the bacteriophage injected at a site distant from the lesion reach the lesion through the circulation and multiply at the expense of the bacteria there?
8. Does the bacteriophage inhibiting substance (antiphage) found in the blood serum of some individuals inhibit the action of bacteriophage when injected into the lesion?
9. Can the apparently favorable effect of bacteriophage treatment in some cases be correlated with the absence of antiphage in the blood, the persistence of bacteriophage in the lesion or any other laboratory finding?

### LITERATURE

The only reports bearing upon these questions which we can find in the recent literature are the following: In the treatment of a small group of twelve cases of urinary infections, Caldwell<sup>4</sup> found that after subcutaneous injection of bacteriophage it could be isolated from the urine. When bacteria disappeared from the urine following bacteriophage therapy the bacteriophage could no longer be recovered from the urine. She noted that the growth and pigment characteristics of the bacteria changed strikingly under bacteriophage action. In those cases in which the urine was not rendered bacteria-free following the use of bacteriophage the bacteria were modified and became resistant. The bacteriophage-resistant bacteria grew in smaller colonies than the original culture.

In 1926, Schumm and Cooke<sup>5</sup> looked for spontaneous bacteriophage in the washings of 40 cases of maxillary sinusitis and found it in four. In 11 of the negative cases, all of which were chronic, 1 c.c. of bacteriophage was instilled at weekly intervals, but no favorable results were obtained, although bacteriophage could subsequently be recovered from a majority of these cases and the organisms cultivated from the lesions remained susceptible to the bacteriophage in the test tube.

In 1926, Epstein and Fygin,<sup>6</sup> employing horse-blood agar, reported that 31 strains of hemolytic staphylococci were resistant to bacteriophage, while 91 nonhemolytic strains were susceptible. McKinley and Camara<sup>7</sup> (1930), working with a bacteriophage which was active against a strain of nonhemolytic staphylococcus, failed in their attempt to adapt it to 7 hemolytic strains. They believed that this observation tended to confirm the work of Epstein and Fygin, and suggested that this might explain the relative virulence of hemolytic organisms. Contrary results, however, were recently obtained by d'Herelle and Rakietsen<sup>8</sup> who studied cultures from a hundred cases of staphylococcus infections, using human and rabbit blood agar. Seven strains of nonhemolytic staphylococci, isolated from chronic cases of furunculosis, sinusitis and osteomyelitis, were resistant to a number of polyvalent bacteriophages, while 71 strains of hemolytic staphylococci, with one exception, were found to be susceptible to the bacteriophage. The remaining 21 nonhemolytic strains were finally lysed, but in several instances only after repeated contact with the bacteriophage. These results were so at variance with the work of the preceding authors that d'Herelle and Rakietsen repeated their experiments with horse-blood agar and found that the hemolytic strains were just as susceptible on that medium as they were on human and rabbit blood agar.

In 1931, Applebaum and McNeal<sup>9</sup> reported that purulent exudates and blood as well as serum, inhibited lysis sufficiently to explain the weakening of the action of bacteriophage and the persistence of living bacteria in purulent lesions treated with bacteriophage.

Colvin in 1932,<sup>10</sup> incidental to his observations on the inhibition of lysis by sterile pus, cerebrospinal fluid and serum, found that bacteriophage could be found in the lesion several days after its administration.

### SOURCE OF MATERIAL AND METHOD OF STUDY

During the course of our study we have worked with four potent staphylococcus bacteriophages obtained from d'Herelle and Rakietsen in New Haven;

from McNeal of the Post-Graduate Hospital, New York City; from Gratia in Brussels; and from Larkum of the Department of Public Health in Lansing, Michigan.

Our bacterial cultures were obtained from 110 patients with furuncles and carbuncles on their first visit to the Vanderbilt Clinic. If the lesion was draining, the exudate was collected on a swab, if not, a small amount of plain broth was injected into its center and reaspirated. This fluid was then cultivated for from three to five hours in plain broth. From the three- to five-hour growth in broth, four 5 c.c. tubes of plain broth were inoculated and to two of these tubes, 0.25 c.c. of bacteriophage was added. The other two tubes were kept as bacterial controls. One tube containing bacteriophage and one control were incubated at room temperature and one of each was incubated at body temperature for twenty-four hours. At the end of this time the tubes inoculated with bacteriophage were read for the degree of lysis and graded from 0 to 4+, the latter grade being assigned only to those tubes which were absolutely clear, comparison being made with the growth in the control tubes.

At the same time that the broth cultures were made, a drop from the control tube and a drop from the tube containing the culture and bacteriophage were smeared on the two halves of a sheep's blood agar plate. If the growth in the control tube at room temperature was less than the growth in the incubated control tube, it was filtered and the filtrate tested for the presence of spontaneous bacteriophage. Next day the plates were studied and the cultural characteristics of the colonies with and without bacteriophage were noted. If the colonies on the control half were rough or varied in size or shape or appeared "moth-eaten," spontaneous bacteriophage was suspected and searched for. When such cultures were found, transplants were made from these colonies into broth, grown at room temperature, filtered, and the filtrate was tested for lysis of the homologous and stock strains. In the more recent phases of our study, we have found spontaneous bacteriophage more often than we did at first because of greater care in the search.

At twenty-four, forty-eight, seventy-two, and ninety-six hours, after bacteriophage was given, cultures were again taken from the lesions and studied for the characteristics of the colonies and the susceptibility of the organisms to bacteriophage. Usually bacteriophage was given twenty-four hours after the first visit, the interval required to determine whether or not the bacteria were susceptible to bacteriophage.

## RESULTS

I. *The Classification of 110 Strains of Staphylococcus Obtained from a Series of Furuncles and Carbuncles.*—In the whole series of 110 cultures, hemolytic *Staphylococcus aureus* occurred 81 times. In some of these cases the hemolysis was slight and could be seen only on removal of the colonies. Nonhemolytic *Staphylococcus aureus* was found in 18, and a mixture of these two in 8. Hemolytic *Staphylococcus albus* was found in one lesion and non-hemolytic *Staphylococcus albus* in one. Hemolytic *Staphylococcus aureus* and nonhemolytic *Staphylococcus albus* were combined in one. The results may be seen in Table I.



TABLE I

THE CLASSIFICATION OF 110 STRAINS OF STAPHYLOCOCCI ACCORDING TO THEIR HEMOLYTIC AND CHROMOGENIC PROPERTIES AND THEIR SUSCEPTIBILITY TO THE LYTIC ACTION OF BACTERIOPHAGE

|                      | NO.<br>OF<br>CULT. | %    | HEMOLYTIC<br>STAPH.<br>AUREUS | NONHEM.<br>STAPH.<br>AUREUS | BOTH<br>HEMOLYTIC<br>&<br>NONHEM.<br>STAPH.<br>AUREUS | HEMOLYTIC<br>STAPH.<br>ALBUS | NONHEM.<br>STAPH.<br>ALBUS | HEMOLYTIC<br>STAPH.<br>AUREUS<br>&<br>NONHEM.<br>S. ALBUS |
|----------------------|--------------------|------|-------------------------------|-----------------------------|-------------------------------------------------------|------------------------------|----------------------------|-----------------------------------------------------------|
| Completely lysed     | 88                 | 80   | 64                            | 16                          | 5                                                     | 1                            | 1                          | 1                                                         |
| Partially lysed      | 19                 | 17.3 | 15                            | 1                           | 3                                                     | —                            | —                          | —                                                         |
| Completely resistant | 3                  | 2.7  | 2                             | 1                           | —                                                     | —                            | —                          | —                                                         |
| Totals               | 110                | 100  | 81                            | 18                          | 8                                                     | 1                            | 1                          | 1                                                         |

II. *The Susceptibility of the Hemolytic and Nonhemolytic Strains of Staphylococcus to Bacteriophage.*—Table I shows that only three of the 110 strains tested were found to be resistant to the action of all of the bacteriophages. Certain of the strains were resistant to one or two, but susceptible to at least one of the bacteriophages which we used. Two of the resistant strains were hemolytic *Staphylococcus aureus* and the other was a nonhemolytic *Staphylococcus aureus*. Of the 81 cultures of hemolytic *Staphylococcus aureus*, 64 were completely lysed; 15 underwent partial lysis; and 2 were resistant. Of the 18 strains of nonhemolytic *Staphylococcus aureus*, 16 underwent complete lysis; one partial lysis; and one was resistant. Of the 8 mixed cultures with both hemolytic and nonhemolytic *Staphylococcus aureus*, complete lysis took place in 5 and partial lysis in 3. With the 3 remaining albus cultures, either alone or with *Staphylococcus aureus*, complete lysis was obtained. In our series, therefore, no difference was found between the hemolytic and nonhemolytic strains in their susceptibility to bacteriophage.

III. *Persistence of Bacteriophage in the Lesion.*—The persistence of the bacteriophage in the lesion was studied in 32 patients, as the opportunity afforded upon their return to the clinic after treatment. In some the search was made after twenty-four hours and in others it was made later. In a few it was made daily, but in most of the cases we desired to institute daily treatment and did not withhold it to study the duration of the bacteriophage following a single injection. These results are shown in Table II. In 20, cultures were taken twenty-four hours after the first administration of potent bacteriophage. As a general rule, these cultures displayed considerable degeneration of the colonies on the blood agar plate. Often only remnants of colonies were left, or the intact colonies varied in size and shape. However, only rarely did we find no growth at all on the plate. On only two occasions, cultures of the lesion twenty-four hours after administration of bacteriophage yielded no growth on the plate or in broth. At the time of these observations, we assumed that bacteriophage was responsible, but its presence was not definitely proved. Broth tubes inoculated with cultures taken twenty-four hours after the administration of bacteriophage usually did not show growth when incubated at room temperature, but gave abundant growth

TABLE II

THE PERSISTENCE OF BACTERIOPHAGE 24, 48, 72, AND 96 HOURS AFTER INJECTION INTO THE LESION CORRELATED WITH THE CLINICAL EVIDENCE OF BENEFIT FOLLOWING THE TREATMENT

| NUMBER   | 24 HR. | 48 HR. | 72 HR. | 96 HR. | CLINICAL EFFECT |
|----------|--------|--------|--------|--------|-----------------|
| 1        |        | +      |        |        | Slight benefit  |
| 2        |        | +      |        |        | Slight benefit  |
| 3        | +      | +      |        |        | Slight benefit  |
| 4        | +      |        |        |        | Marked benefit  |
| 5        | +      | +      |        |        | Marked benefit  |
| 6        | +      |        | 0      |        | Marked benefit  |
| 7        | +      |        |        | 0      | Marked benefit  |
| 8        | 0      | 0      |        |        | No benefit      |
| 9        |        | +      |        | +      | Marked benefit  |
| 10       | +      |        | +      |        | Marked benefit  |
| 11       |        |        |        | +      | Marked benefit  |
| 12       |        |        | +      | 0      | Marked benefit  |
| 13       |        | +      |        |        | Slight benefit  |
| 14       | 0      | 0      |        |        | Marked benefit  |
| 15       | +      | +      | +      |        | Marked benefit  |
| 16       | +      | +      | +      |        | Marked benefit  |
| 17       | +      | +      |        | 0      | Marked benefit  |
| 18       | +      | +      |        |        | Marked benefit  |
| 19       |        | +      |        |        | Marked benefit  |
| 20       | +      | +      |        |        | Slight benefit  |
| 21       | +      |        |        |        | Marked benefit  |
| 22       | +      |        |        |        | Marked benefit  |
| 23       |        |        | +      |        | Marked benefit  |
| 24       | +      |        |        |        | Marked benefit  |
| 25       | +      |        |        |        | Marked benefit  |
| 26       |        | 0      |        |        | No benefit      |
| 27       | 0      | 0      | 0      |        | No benefit      |
| 28       |        | 0      |        |        | Marked benefit  |
| 29       |        | 0      |        |        | No benefit      |
| 30       | 0      |        |        | 0      | No benefit      |
| 31       |        |        | +      |        | Marked benefit  |
| 32       | +      |        |        |        | Marked benefit  |
| Totals   | 20     | 18     | 8      | 6      |                 |
| Positive | 16     | 12     | 6      | 2      |                 |
| Negative | 4      | 6      | 2      | 4      |                 |

in the incubator, although occasionally secondary lysis took place. Filtrates made of the 20 cultures left at room temperature produced complete lysis of the original homologous organisms obtained from the lesion in 16 instances, demonstrating the presence of potent bacteriophage. These bacteriophages often produced lysis of heterologous strains as well. They remained polyvalent and did not become specific for the homologous strain by passage. There is evidence, then, both from the appearance of the blood plates and from the test of the filtrates incubated at room temperature, that bacteriophage may be present in the lesion in about 4 cases out of 5 at least twenty-four hours after a single injection, and that under its influence there is a change in the growth characteristics and morphology of the organisms.

Cultures were taken from 18 cases forty-eight hours after the injection of bacteriophage. In most of these cases the culture was not taken after the first injection of bacteriophage, but later in the course of treatment. Therefore these and later determinations of bacteriophage in the lesion are not as significant as the tests made after twenty-four hours. The characteristics of the colonies on the blood plates made at this interval differed from those taken after twenty-four hours. Usually only a few colonies were af-

fects while the rest were normal. Large areas of destruction in the colonies were seldom observed. Degeneration of the organisms was not seen when these colonies were smeared and stained. Moreover, in the tubes incubated at room temperature some growth of organisms occurred in a high percentage of cases. When these tubes were filtered, however, the lytic principle was obtained from 12 of the 18, although the potency of the bacteriophage was definitely diminished. Those incubated at 37° C. usually showed abundant growth of bacteria.

It must be concluded, then, that bacteriophage persisted in the lesions for forty-eight hours in a smaller percentage of the cases and had less potency than at twenty-four hours.

Bacteriophage persisted in the lesion in 6 out of 8 cases tested after seventy-two hours, and in 2 out of 6 tested after ninety-six hours. The colonies on the blood plates showed no evidence of the destructive action, but the bacteriophages were recovered from the filtrates of cultures incubated at room temperature. These bacteriophages frequently gave only partial lysis, however.

IV. *The Relationship of the Persistence of Bacteriophage in the Lesion to the Presence of Antiphage in the Blood.*—In 22 cases we tried to correlate the persistence of bacteriophage in the lesion with the presence of an inhibiting substance in the blood serum. In testing for the presence of this substance we employed Rosenthal's technic.<sup>11</sup> In some instances the antiphage tests were done with the homologous strain of bacteria and in other cases with stock strains (Table III). Indirect antiphage (supposed to render the bacteria resistant to the lytic action of the bacteriophage) was only detected in 3 cases, while the direct antiphage (supposed to directly neutralize the action of the bacteriophage) was present in 16. Of 10 cases in which the test was made with the homologous strain, 7 showed a direct antiphage in the blood, but in 6 of these cases bacteriophage was found in the lesion from twenty-four to ninety-six hours after injection. The other 3 cases showed no direct antiphage in the blood, and bacteriophage was found in the lesion.

Of 11 cases in which the test was made for indirect antiphage using the homologous strain, two showed the presence of antiphage in blood, but the bacteriophage was later found in the lesion in both cases. In 9 cases no antiphage was found, but in 2 of these no bacteriophage could be found in the lesion.

Of 11 cases in which the test was made for direct antiphage, using a heterologous strain, 9 showed the presence of antiphage in the blood and yet in 7 of these bacteriophage was found in the lesion. In the 2 cases in which no antiphage was found in the blood, bacteriophage was present in the lesion. In the same 11 cases when the test was made for indirect antiphage using a heterologous strain, one showed the presence of antiphage in the blood, but bacteriophage was found in the lesion after twenty-four hours, while in 2 cases in which no antiphage was found in the blood, no bacteriophage could be found in the lesion after twenty-four hours. In 8 cases no antiphage was demonstrated in the blood and bacteriophage persisted in the lesion.

TABLE III

THE PRESENCE OF A BACTERIOPHAGE-INHIBITING SUBSTANCE, "ANTIPLHAGE," IN THE BLOOD SERUM OF THE PATIENT CORRELATED WITH THE PERSISTENCE OF BACTERIOPHAGE IN THE WOUND FOLLOWING TREATMENT

| NO. | ANTIPLHAGE IN THE SERUM* |         |          | PERSISTENCE OF PHAGE IN THE LESION |          |          |
|-----|--------------------------|---------|----------|------------------------------------|----------|----------|
|     | STRAIN TESTED            | DIRECT  | INDIRECT | 24 HOURS                           | 48 HOURS | 72 HOURS |
| 1   | Homol.                   | Strong  | Weak     | +                                  | -        |          |
| 2   | Homol.                   | 0       | 0        | +                                  |          |          |
| 3   | Homol.                   | Weak    | 0        | +                                  |          |          |
| 4   | Homol.                   | No test | 0        | 0                                  | 0        |          |
| 5   | Homol.                   | Strong  | 0        |                                    | +        |          |
| 6   | Homol.                   | Strong  | 0        | +                                  | +        |          |
| 7   | Homol.                   | Strong  | 0        | +                                  | +        | +        |
| 8   | Homol.                   | 0       | 0        |                                    | +        | +        |
| 9   | Homol.                   | Strong  | 0        |                                    | 0        |          |
| 10  | Homol.                   | 0       | 0        | +                                  | +        |          |
| 11  | Homol.                   | Weak    | Weak     |                                    |          |          |
| 12  | Heterol.                 | Strong  | 0        |                                    | +        | +        |
| 13  | Heterol.                 | Strong  | 0        | +                                  |          | 0        |
| 14  | Heterol.                 | Weak    | 0        | +                                  | +        |          |
| 15  | Heterol.                 | Strong  | 0        |                                    |          | +        |
| 16  | Heterol.                 | Weak    | 0        | 0                                  | 0        | 0        |
| 17  | Heterol.                 | Strong  | 0        |                                    | +        |          |
| 18  | Heterol.                 | Strong  | 0        |                                    |          |          |
| 19  | Heterol.                 | 0       | 0        | +                                  |          |          |
| 20  | Heterol.                 | Strong  | Strong   | +                                  | +        |          |
| 21  | Heterol.                 | 0       | 0        |                                    |          |          |
| 22  | Heterol.                 | Weak    | 0        | +                                  | +        |          |

\*As determined by the Rosenthal technic.

V. *Transmission of Bacteriophage in the Patient from a Site Remote from the Lesion to the Lesion Itself.*—In 8 patients, satellite furuncles developed around the original lesion treated with bacteriophage. Attempts to isolate the bacteriophage from these new lesions failed. Again, 4 patients were given subcutaneous injections of large quantities of bacteriophage at a distance from the lesion and bacteriophage could only be isolated from one of the lesions twenty-four hours later. This bacteriophage was very weak, producing only 1+ lysis of the homologous organism, and it produced no lysis of two heterologous strains against which the injected bacteriophage had previously been effective. In this one case, no bacteriophage was evident in the lesion before the injection, so that we must presume that it had either been transmitted from the site of the injection or was spontaneously generated. With the exception of this one questionable instance, we have no evidence that bacteriophage can be transmitted within the body from a site distant from a skin lesion to the lesion itself.

VI. *Behavior of the Bacteria Which Survive in the Lesions Which Have Been Treated with Bacteriophage.*—Our experience has shown that the surviving progeny of a culture of hemolytic *Staphylococcus aureus* which has been lysed by bacteriophage but not entirely destroyed, produces nonhemolytic colonies when grown on blood agar plates. Usually the colonies at first become mixed, some being hemolytic and some nonhemolytic, but in a few instances in our series all of the colonies promptly became completely non-hemolytic. In vivo a similar change has occasionally been observed, but it required more prolonged contact of the bacteria with the bacteriophage. Of

23 cases studied, this transformation occurred in 7. In 5 of these, the hemolytic variety was replaced after one or more bacteriophage treatments by a mixture of the hemolytic and nonhemolytic strains. In another, the hemolytic strain was completely replaced by nonhemolytic organisms, while in the last case the culture yielded a mixture of hemolytic *Staphylococcus aureus* and nonhemolytic *Staphylococcus albus*. The possibility of contamination in this case is recognized.

We have never observed a transformation of the staphylococcus from nonhemolytic to hemolytic after lysis in the test tube by bacteriophage, but such a change was observed on three occasions in patients in the course of bacteriophage treatment. Two of these new strains became resistant to bacteriophage, whereas the original cultures were completely lysed before treatment began.

These cases also illustrate that bacteria may become resistant to the bacteriophage during treatment. Acquisition of resistance was observed in 6 out of 36 cases, and in 4 of these, cultures were taken as the lesions were healing. In 8 instances the bacteria became more susceptible to bacteriophage while in the remaining 22 cases no change in the susceptibility of the bacteria to the bacteriophage took place.

It was also observed, after bacteriophage had been injected into the lesion, that the colonies frequently changed from smooth to rough. Moreover, some of the colonies became pinpoint. No further changes than these could be detected in their characteristics as healing progressed in the presence of bacteriophage, except that some of the colonies from the larger carbuncles changed in color from yellow to white.

At no time could we surely demonstrate a complete destruction of bacteria in the lesion tested with repeated injections of bacteriophage. They persisted even after the slough and pus had disappeared. On several occasions, moreover, positive cultures were obtained after the removal of the terminal scab.

Bacteria also persisted in 7 lesions in which bacteriophage was generated spontaneously. These cultures showed considerable degeneration, however, and many of the colonies were roughened. All of the bacteriophages isolated from these lesions were polyvalent and possessed considerable potency.

From our results, therefore, we can only conclude that while the bacteriophage may change the characteristics of the bacteria in the lesion, it certainly does not completely destroy them.

VII. *The Correlation of Various Laboratory Findings With the Clinical Results.*—Untreated furuncles and carbuncles run a varied course under the variable conditions found in different individuals. Furuncles tend to be self-limited. There is a period of induration, a period of liquefaction and discharge, and a period of resolution. The same periods are more prolonged in carbuncles. The extent of the slough, the rapidity of liquefaction, and the size and number of the drainage openings all modify the period of spontaneous healing. With such variable controls it is very difficult to judge in any given cases whether or not any form of treatment hastens or delays the process of

healing. In order to furnish convincing evidence, we must await the acquisition of a large series of treated cases with an exactly parallel series of controls, unless we can apply a clear-cut laboratory criterion. This we are trying to establish. For the present we have tried to note from the appearance of the lesion after treatment, as well as from the subjective symptoms of the patient, whether or not the progress of the infection indicated a favorable or an indifferent effect from the bacteriophage treatment. (In no case have we had an unfavorable reaction.) We have tried to discover whether this could be correlated with (a) the presence of a bacteriophage-inhibiting substance, "antiphage," in the blood, or (b) the persistence of bacteriophage in the lesion, or (c) the maintenance of susceptibility to the bacteriophage on the part of the invading organism, or (d) changes in the hemolytic action of the cultures on blood agar.

(a) *Antiphage*: In 34 cases a study was made of the presence of antiphage in the blood with the Rosenthal technic,<sup>11</sup> and the tests were carried out with the homologous organisms (see Table IV). Eight of these cases gave evidence of the presence of both strong direct and strong indirect antiphage. After instituting bacteriophage treatment, our belief was that the bacteriophage had exerted a favorable influence in 5 of these and had had no influence on the infection in 3. Seven of the cases gave a strongly positive test for direct and a negative test for indirect antiphage. Of these, 5 gave a favorable response to bacteriophage, while 2 seemed to be slightly improved by it. Eight of the cases gave evidence of a weak direct antiphage and no sign of indirect antiphage in the blood; and of these, 5 gave a favorable response and 2 a questionable response, while one was not affected. Five cases gave no evidence of direct or indirect antiphage. Of these, 2 were favorably affected and 3 gave a questionable response. The other groups are too small to be significant.

TABLE IV

THE PRESENCE OF A BACTERIOPHAGE-INHIBITING SUBSTANCE IN THE BLOOD SERUM OF THE PATIENT CORRELATED WITH THE CLINICAL EFFECT OF PHAGE TREATMENT

| NO. OF<br>CASES | ANTIPHAGE IN SERUM* |      |      |          |      |      | CLINICAL EFFECT OF PHAGE<br>TREATMENT |                   |               |
|-----------------|---------------------|------|------|----------|------|------|---------------------------------------|-------------------|---------------|
|                 | DIRECT              |      |      | INDIRECT |      |      | BENEFIT                               | SLIGHT<br>BENEFIT | NO<br>BENEFIT |
|                 | STRONG              | WEAK | NONE | STRONG   | WEAK | NONE |                                       |                   |               |
| 8               | +                   |      |      | +        |      |      | 5                                     | 0                 | 3             |
| 3               | +                   |      |      |          | +    |      | 2                                     | 1                 | 0             |
| 7               | +                   |      |      |          |      | +    | 5                                     | 2                 | 0             |
| 0               |                     | +    |      | +        |      |      | 0                                     | 0                 | 0             |
| 1               |                     | +    |      |          | +    |      | 1                                     | 0                 | 0             |
| 8               |                     | +    |      |          |      | +    | 5                                     | 2                 | 1             |
| 0               |                     |      | +    | +        |      |      | 0                                     | 0                 | 0             |
| 2               |                     |      | +    |          | +    |      | 2                                     | 0                 | 0             |
| 5               |                     |      | +    |          |      | +    | 2                                     | 3                 | 0             |

\*As determined by Rosenthal's technic.

(b) *Persistence of Bacteriophage in the Lesion* (see Table II): In the 32 cases in which the bacteriophage was sought for, twenty-four to ninety-six hours after its injection into the lesion, 16 cases out of 20 tested showed the bacteriophage in twenty-four hours. Of these, 14 gave a very favorable re-

sponse while 2 were slightly favorable. Of 4 cases which yielded no bacteriophage after twenty-four hours, one seemed to be favorably affected by the treatment while 3 were unaffected. The favorable case had already completely liquefied when treatment began. In forty-eight hours, 18 cases were tested for the persistence of bacteriophage. Twelve of these cases showed bacteriophage in the lesion at that time. Seven of them responded very favorably, while in 5 the effect was slightly favorable. Of 6 cases which showed no bacteriophage after forty-eight hours, 2 seemed to respond well after bacteriophage treatment, while 4 gave no evidence of any favorable response. One of the favorable cases had already liquefied. Out of 8 cases tested after seventy-two hours, bacteriophage was found in 6 and all seemed to be benefited by the bacteriophage treatment. In the 2 cases in which no bacteriophage was found after seventy-two hours, one seemed to be favorably affected by bacteriophage treatment while the other was not. In the favorable case, bacteriophage had been found in twenty-four hours. In 6 cases bacteriophage was sought for, ninety-six hours after its injection. It was found in 2 cases only. In 3 others it had been present at twenty-four, forty-eight, or seventy-two hours. In all of these cases, the result was favorable. In the other case in which it was not found, it had also been absent at forty-eight hours. In this case the effect of bacteriophage was negligible.

(c) *Maintenance of Susceptibility of the Bacteriophage on the Part of the Culture:* After treatment with bacteriophage, as we have stated above, the organism cultured from the lesion may show an increased or decreased susceptibility to the bacteriophage. This observation was correlated with the clinical response in twenty-eight cases (see Table V). In 25 of these cases the organism remained susceptible to the bacteriophage. Of these, 20 gave a favorable response while 5 were not benefited. In 2 cases the organism was only partially susceptible and these cases were not benefited. In 3 cases the organism became resistant to the bacteriophage and none of these was helped by the bacteriophage treatment.

TABLE V

THE MAINTENANCE OF SUSCEPTIBILITY TO PHAGE ON THE PART OF THE CULTURE CORRELATED WITH THE CLINICAL EFFECT OF BACTERIOPHAGE TREATMENT

|                      | NO. OF<br>CASES | CLINICAL EFFECT OF PHAGE<br>TREATMENT |            |
|----------------------|-----------------|---------------------------------------|------------|
|                      |                 | BENEFITED                             | NO BENEFIT |
| Remained susceptible | 25              | 20                                    | 5          |
| Became resistant     | 3               | 0                                     | 3          |

(d) *The Changes in the Hemolytic Action of the Culture on Blood Agar:* We have stated above that when hemolytic *Staphylococcus aureus* is lysed by bacteriophage in the test tube, if it is not entirely destroyed, the progeny are generally nonhemolytic. These bacteria are evidently resistant to the lytic effect of the bacteriophage. In treated cases these forms often appear, but not as regularly as in the test tube. The clinical result in these cases is not as favorable as in those in which the organism, which is recovered from

the lesion after treatment, is the same as it was before treatment whether it were hemolytic or nonhemolytic. In thirty-four cases in which this point was studied, 22 showed no change in the culture and the clinical results were favorable in 18 and indifferent in 4. In the other 12 cases there was some modification of the culture, consisting of a change either in the hemolytic or chromogenic function of the organism. In 4 of these the results were favor-

TABLE VI

CHANGES IN THE CULTURAL CHARACTERISTICS OF THE BACTERIAL STRAINS FOLLOWING BACTERIOPHAGE TREATMENT CORRELATED WITH THE CLINICAL RESULTS

|                      | NO. OF<br>CASES | CLINICAL EFFECT OF PHAGE TREATMENT |                   |               |
|----------------------|-----------------|------------------------------------|-------------------|---------------|
|                      |                 | BENEFITED                          | SLIGHT<br>BENEFIT | NO<br>BENEFIT |
| No change in culture | 22              | 18                                 | 0                 | 4             |
| Change in culture    | 12              | 4                                  | 2                 | 6             |

able, in two there was partial success, either favorable at first and later indifferent, or indifferent at first and later favorable, while in 6 there was no apparent response to the treatment (see Table VI).

#### DISCUSSION

We recognize the difficulty of judging whether or not the administration of bacteriophage affects in any way the course of the disease. In order to be thoroughly convinced by clinical criteria alone, we should have two absolutely parallel series, one treated with bacteriophage and one untreated. We are at present attempting to assemble two such parallel series, taking into account the age of the lesion, its location, the presence and degree of necrosis, and the size of the lesion at the time of the beginning of treatment. If the results in these treated cases can be correlated with certain consistent laboratory findings, the latter may be depended upon as bases for prognosis.

It has been observed repeatedly that the most severe staphylococcus infections generally yield on culture a hemolytic strain of *Staphylococcus aureus*. Lesions somewhat less severe may yield a culture showing no hemolysis or a very slight degree of hemolysis beneath a few colonies on a blood plate. Still less severe lesions more often yield *Staphylococcus albus* rather than *S. aureus*. In our series the primary cultures yielded the usual proportion of such variable strains. The cultures which we called a mixture of hemolytic and nonhemolytic *Staphylococcus aureus* may have been pure cultures of the intermediate group, but usually those showing frankly yellow and frankly white colonies do represent a mixture of aureus and albus. It is rather surprising that in lesions of this kind mixtures are not more frequently found.

In our studies the hemolytic and nonhemolytic strains showed an equal susceptibility to the bacteriophages which we employed. Attention must be called to the fact, however, that our organisms were freshly isolated from acute staphylococcus infections and the same results might not have been obtained with staphylococci obtained from chronic infections or with cultures grown for some time in artificial media. Thus we did not confirm the observations of either d'Herelle and Rakieten or McKinley and Camara whose results



were opposed to one another. Differences in results could probably be explained by differences in the bacteriophages employed. This point does not seem to be important. There does seem to be some significance, however, in the fact that both in the test tube and in the patient the organisms undergo certain changes after contact with the bacteriophage. The hemolytic strains frequently become nonhemolytic, the smooth strains tend to become rough, and while they may become more resistant to the lytic action of the bacteriophage, both of these changes are in the direction of nonvirulence. It should be remembered that this occurred more consistently in the test tube than in the patient, but such changes occurring in the lesion might favor the prompt recovery from the infection by the action of the defensive forces of the body.

The persistence of the bacteriophage for twenty-four hours or more in the lesion after one injection is of considerable importance from a practical viewpoint. We do not feel that there is any evidence that it actually propagates itself there, and when injected elsewhere what may appear in the lesion gives no evidence of increasing either in quantity or in potency. In the lesion evidently something inhibits the multiplication of the bacteriophage, and yet it is not due to the presence of any inhibiting substance in the patient's serum, if Rosenthal's method can be relied upon, for the failure of the bacteriophage to persist in the lesion does not run parallel with the presence of such a substance in the blood as shown by this test. If Rosenthal and Raiga obtained better results in their cases by autohemotherapy it is hard to believe that this fact is due to any alteration of this substance in the blood. The failure to corroborate their results has induced us to study this phase of the problem more fully. The failure of the bacteriophage, recovered from the wound either after twenty-four or forty-eight hours, to act at incubator temperature would seem to indicate that it is less potent than the original bacteriophage, and it is, therefore, overwhelmed by the growth of the organisms at the temperature most suitable for their propagation. This temperature is, of course, the temperature at which bacteria grow in the body, and therefore, the inaction of the bacteriophage at body temperature may be of some significance.

The weakening of the bacteriophage by its habitation in the lesion for twenty-four hours was also indicated by the development of secondary growth after lysis. With the potent bacteriophages originally employed, secondary culture formation seldom occurred, but with the bacteriophages obtained from the lesion, either those arising spontaneously or those persisting for a day or two after instillation, secondary growth was the rule.

All of these results fail to support the idea that the bacteriophage propagates itself in the lesion. This points to the necessity of frequent reinoculations of the bacteriophage into the lesion. If bacteriophage is not reinjected into the lesion, at the end of twenty-four hours the growth of bacteria untouched by the original injection may continue unabated. These facts need further elucidation, but in the meantime there is a very definite indication for the frequently repeated administration of large quantities of bacteriophage, if it is to be used at all. Until we know what is inhibiting its propa-

gation in the body or in the lesion we must expect very little action following its injection at a distance from the lesion, except whatever may be gained from the injection of the lytic products of the bacteria.

In attempting to correlate the clinical results with certain laboratory findings, it would appear from the above observations that the presence or absence of antiphage in the blood as determined by Rosenthal's method has little or no effect on the usefulness of bacteriophage treatment. This point will be studied further and enlarged upon in another paper. However, the persistence of bacteriophage in the lesion and the persistence of susceptibility of the culture to the bacteriophage, as well as the absence of the development of progeny having cultural characteristics different from the original culture, are all factors of considerable importance. These three may all be important because they may indicate the potency of the bacteriophage. If there can be any other simple laboratory method of determining the potency of bacteriophage and maintaining its potency, the problem will be greatly simplified. But the efficiency of any treatment is determined to a great degree by the phase of the disease in which the treatment is begun. This is particularly true of infections for even after the infection is under control the process of healing is greatly modified by the kind and extent of the tissues which have been destroyed.

#### SUMMARY

One hundred and ten cases of furuncles and carbuncles have been studied from the point of view of the cultural characteristics of the strains of staphylococcus obtained from them and the susceptibility of those strains to the lytic action of bacteriophage as well as the changes in the cultural characteristics of these strains both in the test tube and in the lesion following the repeated administration of the bacteriophage. Certain features of the behavior of the bacteriophage and its persistence in the lesion have also been studied, and an attempt has been made to correlate the persistence of the bacteriophage or the disappearance of it in the lesion with the absence or presence of an inhibiting substance in the patient's serum known as antiphage. Our findings were briefly as follows:

1. A relationship could not be demonstrated between the hemolytic or nonhemolytic properties of the staphylococci and their susceptibility to bacteriophage. Of the 110 strains tested, only three were resistant. In the whole series, 81 were hemolytic *Staphylococcus aureus* and 18 were nonhemolytic *Staphylococcus aureus*. The rest were intermediate or mixed strains. These tests were all made with freshly isolated strains of bacteria and with several potent bacteriophages. The results indicate that if potent bacteriophages are employed practically all strains of staphylococci from acute lesions can be lysed.

2. The bacteria in the lesions were found to be altered in their cultural characteristics following the repeated injections of bacteriophage. After twenty-four hours the colonies on the blood plates showed degenerative changes, areas of absent growth, and "a moth-eaten appearance." At forty-eight hours these changes were not as marked, although after more injections, the colonies sometimes became roughened and pinpoint in size. A few

changed in their chromogenic properties and some in their hemolytic properties. Some became more resistant to bacteriophage and still others less resistant. In the majority of instances, the cultures remained unchanged in their susceptibility to bacteriophage. It was seldom demonstrated that the lesions became absolutely free of bacteria either after repeated injections of bacteriophage or when the bacteriophage was generated spontaneously in the lesion, although in two cases negative cultures were obtained both on the plate and in broth. This may have been due to inhibition by bacteriophage rather than actual destruction, for subsequent cultures were positive.

3. After one injection of bacteriophage, the lytic principle could be re-isolated from the lesion at the end of twenty-four hours and forty-eight hours. At these intervals the bacteria showed evidence of destruction, particularly at the twenty-four-hour period. The bacteriophage could also be recovered from the filtrate of cultures incubated at room temperature after seventy-two and ninety-six hours, after instillation into the lesion, but in a much weakened state. While the bacteriophage persisted in the lesion and still had some destructive action after twenty-four hours, it did not increase in potency as it did in the test tube. Instead, it grew progressively weaker.

4. In about a third of these cases, the serum of the patients was tested for the presence of "antiphage," a substance which has the power to inhibit the action of bacteriophage in the test tube. This principle was found in the blood serum of a number of these patients, but there was no correlation between this fact and the persistence or disappearance of bacteriophage in the lesion.

5. The favorable clinical effect of the use of bacteriophage seems to have some correlation with the persistence of bacteriophage in the lesion and the maintenance of the cultural characteristics and susceptibility of the strain producing the disease and may have little or nothing to do with the presence or absence of the so-called "antiphage" (bacteriophage inhibiting substance) in the blood serum of the patient.

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# CORONARY OCCLUSION AND SUDDEN DEATH

A STUDY BASED UPON A REVIEW OF 345 NECROPSIES PERFORMED AT THE BOARD OF HEALTH LABORATORY, ANCON, CANAL ZONE, BETWEEN APRIL 15, 1932, AND APRIL 15, 1933

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## HISTORY

CORONARY occlusion was recognized and described at autopsy by pathologists forty to fifty years before wide medical interest was aroused in the disease about 1918. Coronary disease "has only been generally recognized and found to be extremely common since about 1926," according to Rolleston,<sup>1</sup> who also states that the condition was first described adequately by Weigert in 1880, but that a clinical diagnosis of coronary thrombosis was made in 1876 by Adam Hammer. In a general review, Benson<sup>2</sup> traces first observations on coronary sclerosis to Drelinecourt in 1700. Dock recognized the disease during life in one patient, and in 1896 reported the case with autopsy confirmation.<sup>3</sup> In 1910, in Germany, two Russians, Obrastzow and Straseshesko<sup>4</sup> adequately described the clinical syndrome. Herrick<sup>5</sup> soon described the disease in America. Osler<sup>6</sup> early correlated the clinical and pathologic findings; he believed that the disease was one which affected mainly the upper classes, and he used as examples, John Hunter, Charcot, Nothnagel, and William Pepper, each of whom died of coronary disease.

## CASE REPORTS

There are a few general considerations to be mentioned before reviewing the cases listed in Table I. A constriction of 95 per cent or more of the arterial lumen is considered as occlusion in this paper. All autopsies were performed within twenty-four hours postmortem, and included examination of the brain, microscopic examination of all organs, and except in embalmed bodies, serologic and chemical examination of the blood and spinal fluid. In the embalmed bodies fixing fluid generally was flowing into the right common carotid artery within three hours after death. Cases 1 to 11, inclusive, are of coronary sclerosis; Cases 12 to 15, inclusive, are of coronary sclerosis with thrombosis; Cases 16 and 17 are of constriction of the coronary orifice by syphilitic aortitis; and Case 18 is of coronary embolism from a mitral valve vegetation.

## DISCUSSION

*Age.*—As shown in Table II, the fifth and sixth decades claimed the most cases. This frequency is one decade younger than the usual published figures,

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and may be explained by the relatively younger ages of the inhabitants of the Zone. However, this is not a disease of the aged as shown by Conner and Holt,<sup>7</sup> who in a study of 287 cases found that initial symptoms occurred before fifty-one years of age in 33 per cent and before sixty-one years of age in 75 per cent. According to Eggleston<sup>3</sup> the disease is rare before forty, not uncommon between forty and fifty, and most frequent between fifty and seventy. The ages of the embolic and of the syphilitic constriction of the orifice cases may be much younger. The age of a black female who recently died suddenly from syphilitic atresia of the left coronary orifice was sixteen years.

*Sex.*—There were many more males (87.5 per cent) than females (12.5 per cent), and since in the Canal Zone all cases of sudden death are sent to the morgue for autopsy these figures probably represent the existing situation. Published series show a variation from about 10 to 30 per cent in the number of females affected.

*Race.*—Although in autopsies on 286 bodies over one year of age only 78 were white, the number of coronary sclerosis deaths was exactly twice as numerous in the white as in the black race. Considering all types of coronary occlusion there was a marked preponderance in the white race, the usual finding.

*Symptoms.*—The symptoms are best demonstrated by the four hospitalized cases (2, 8, 10 and 14), three of which showed sclerosis only and one of which showed sclerosis with thrombosis. Dyspnea occurred in all four cases. Cardiac pain, gastric symptoms (of nausea, vomiting, or epigastric discomfort), fever (because of the profuse sweating the oral temperature reading may be below normal, but a rectal temperature reading will generally indicate the presence of fever) and/or cough occurred in three. In two cases tachycardia, low blood pressure and leucocytosis were found. Enlarged heart, pericardial friction rub, and albuminuria were found once. The electrocardiogram was used once and correctly aided in the diagnosis. It is interesting to note that in a study of coronary occlusion at Peter Bent Brigham Hospital in 1923, Wearn<sup>8</sup> stated that there was "no typical or characteristic electrocardiographic curve of the condition." The clinical symptoms are due to the ischemic necrosis of the myocardium and they are well described in the literature,<sup>9</sup> and in recent textbooks. Hamman<sup>10</sup> has collected the symptoms into four groups based upon the pathologic changes, as follows: (1) The immediate symptoms associated with the lesion, the anginal seizure; namely, pain and shock manifested by prostration, fall in blood pressure, and suppression of urine; (2) the symptoms associated with the myocardial change; namely, dyspnea, feeble cardiac impulse, and chronic passive congestion manifested by cyanosis, pulmonary edema, enlarged liver, and albuminuria; (3) the symptoms associated with myocardial infarction; namely, fever and leucocytosis, pericarditis, and embolic phenomena; and (4) additional symptoms such as gastric, vasomotor and nervous.

The longest duration of symptoms before death in sclerosis was three and one-half years (Case 2), and then the patient died suddenly. One patient had symptoms for six months; 2 had symptoms for two months; and 2 had some previous but slight symptoms. In 3 the duration of symptoms is unknown. Since the mortality of the first attack is only about 16 per cent,<sup>7</sup> prompt recogni-

TABLE I

| CASE | AUTOPSY NUMBER | AGE | SEX | COLOR | CLINICAL RECORD                                                                                                                                                                                           | HEART  |             |                          |   | OTHER ARTERIO-SCLEROSIS | GENERALIZED PASSIVE CONGESTION | REMARKS                                                 |
|------|----------------|-----|-----|-------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------------|--------------------------|---|-------------------------|--------------------------------|---------------------------------------------------------|
|      |                |     |     |       |                                                                                                                                                                                                           | WEIGHT | MYOFIBROSIS | ACUTE GROSS DEGENERATION |   |                         |                                |                                                         |
| 1    | 9778           | 44  | M   | B     | Several attacks of precordial pain and dyspnea last 6 mo. Sudden attack 3 days before death of pain, nausea, dyspnea, prostration.                                                                        | 740    | +           | +                        | + | +                       | +                              | Microscopic luetic aortitis.                            |
| 2    | 9787           | 55  | M   | W     | Epigastric pain, dyspnea, palpitation, hyperacidity called gastritis 3 years before death. Hospitalized 10 days for anorexia and gastric discomfort, suddenly fell dead. B.P. 103/54. Normal temperature. | 335    | +           | +                        | + | +                       | +                              | Embalmed body.                                          |
| 3    | 9818           | 53  | F   | W     | Hospitalized 4 yr. Involuntary psychosis; hypertension. Fever and prostration last 2 days.                                                                                                                | 280    | +           | -                        | + | +                       | +                              | Albuminuria. Microscopic myocardial infarction.         |
| 4    | 9826           | 56  | M   | W     | Sudden substernal discomfort at night; fell dead coming from bathroom.                                                                                                                                    | 535    | -           | +                        | + | +                       | -                              | Embalmed body. No head exam.                            |
| 5    | 9845           | 40  | M   | W     | Sudden epigastric and respiratory distress and death while fishing.                                                                                                                                       | 450    | -           | +                        | + | +                       | +                              | Embalmed body.                                          |
| 6    | 9921           | 59  | M   | B     | Arose from sleep, went to bathroom, fell dead.                                                                                                                                                            | 445    | +           | +                        | + | +                       | +                              |                                                         |
| 7    | 9972           | 50  | M   | W     | Sudden nausea and death following sexual excitement.                                                                                                                                                      | 450    | +           | +                        | + | +                       | +                              | Embalmed body.                                          |
| 8    | 9994           | 72  | M   | B     | In Leper Colony 12 yr. Acute abdominal pain 2 mo. before death with fever, cough, tachycardia, hemoptysis, albuminuria, anasarca.                                                                         | 690    | +           | +                        | + | +                       | +                              | Syphilitic aortitis. Wassermann four-plus.              |
| 9    | 10059          | 45  | M   | B     | Sudden acute respiratory distress and substernal pain. Death within half hour. Similar but mild and transient attacks previously.                                                                         | 425    | +           | +                        | + | -                       | +                              | Coronary constriction of only 80%. Syphilitic aortitis. |
| 10   | 10088          | 44  | M   | B     | Sudden nausea and vomiting after lunch. B.P. 94/64; T. 96.8° F.; sweating; W.B.C., 10,000; polys. 78%. Death within 12 hr.                                                                                | 460    | -           | +                        | + | +                       | +                              |                                                         |

TABLE I—Cont'd

| TABLE I—CONT'D |                |     |     |       |                                                                                                                                                                                              |        |             |                          |   |                         |                                |                                                                                   |
|----------------|----------------|-----|-----|-------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------------|--------------------------|---|-------------------------|--------------------------------|-----------------------------------------------------------------------------------|
| CASE           | AUTOPSY NUMBER | AGE | SEX | COLOR | CLINICAL RECORD                                                                                                                                                                              | HEART  |             |                          |   | OTHER ARTERIO-SCLEROSIS | GENERALIZED PASSIVE CONGESTION | REMARKS                                                                           |
|                |                |     |     |       |                                                                                                                                                                                              | WEIGHT | MYOFIBROSIS | ACUTE GROSS DEGENERATION |   |                         |                                |                                                                                   |
| 11             | 10108          | 57  | M   | W     | Awakened by sudden heart pain; respiratory distress and death within half hour. No previous symptoms.                                                                                        | 350    | +           | +                        | + | +                       | +                              | No albuminuria                                                                    |
| 12             | 9815           | 57  | M   | W     | Suddenly fell dead at home.                                                                                                                                                                  | 385    | +           | +                        | + | +                       | +                              | Embalmed body                                                                     |
| 13             | 9926           | 63  | M   | W     | Awakened by coughing spell. Dead within one hour. Previous moderate cough and fluttering heart.                                                                                              | 395    | +           | +                        | + | +                       | +                              | Embalmed body                                                                     |
| 14             | 9988           | 46  | M   | W     | Hospitalized 8 days before death with sudden precordial pain. Fever; tachycardia; B.P. 130/90; W.B.C. 7,400; polys. 71%; typical E.K.G. Pericardial friction rub 2 days before death. Cough. | 450    | +           | +                        | + | +                       | +                              | Embalmed body. Endocardial thrombus. Fibrinous pericarditis. No head examination. |
| 15             | 10100          | 62  | M   | W     | Recently married. Died suddenly in bathroom. Slight indigestion for last 3 mo.                                                                                                               | 535    | +           | +                        | + | +                       | +                              | Cholelithiasis                                                                    |
| 16             | 9871           | 36  | M   | B     | Sudden collapse and death during first hour's work in morning. No previous symptoms.                                                                                                         | 410    | +           | +                        | + | +                       | +                              | Atresia of anomalous coronary orifice by syphilitic aortitis.                     |
| 17             | 9916           | 58  | M   | B     | Collapsed while walking; death within an hour.                                                                                                                                               | 480    | +           | +                        | + | +                       | +                              | Microscopic myocardial infarction. Syphilitic aortitis. Wassermann four-plus.     |
| 18             | 9797           | 26  | F   | B     | Four months endocarditis lenta. Sudden prostration 40 hr. before death; B.P. 90/65; sweating; respiratory distress.                                                                          | 570    | +           | +                        | + | +                       | +                              | Subacute bacterial endocarditis, mitral. Terminal pericarditis.                   |

TABLE II  
TYPES OF CORONARY OCCLUSION

|              | CORONARY<br>SCLEROSIS<br>WITHOUT<br>THROMBOSIS | CORONARY<br>SCLEROSIS<br>WITH<br>THROMBOSIS | SYPHILITIC<br>ATRESIA OF<br>CORONARY<br>ORIFICE | CORONARY<br>EMBOLISM | TOTALS |
|--------------|------------------------------------------------|---------------------------------------------|-------------------------------------------------|----------------------|--------|
| No. of Cases | 11                                             | 4                                           | 2                                               | 1                    | 18     |
| Age 26       | 0                                              | 0                                           | 0                                               | 1                    | 1      |
| 36           | 0                                              | 0                                           | 1                                               | 0                    | 1      |
| 40-47        | 4                                              | 1                                           | 0                                               | 0                    | 5      |
| 50-59        | 6                                              | 1                                           | 1                                               | 0                    | 8      |
| 62-63        | 0                                              | 2                                           | 0                                               | 0                    | 2      |
| 72           | 1                                              | 0                                           | 0                                               | 0                    | 1      |
| Sex          |                                                |                                             |                                                 |                      |        |
| Male         | 10                                             | 4                                           | 2                                               | 0                    | 16     |
| Female       | 1                                              | 0                                           | 0                                               | 1                    | 2      |
| Race         |                                                |                                             |                                                 |                      |        |
| White        | 6                                              | 4                                           | 0                                               | 0                    | 10     |
| Black        | 5                                              | 0                                           | 2                                               | 1                    | 8      |

tion is an advantage for earlier and prolonged rest treatment and a better functional outlook.

*Pathology.*—Unfortunately “coronary thrombosis and infarction of the heart are generally spoken of in the same breath as if they were one and the same thing,”<sup>12</sup> whereas ischemic necrosis of the myocardium may result from only an extreme degree of narrowing of the coronary arteries, a lesion too seldom stressed in the literature. That “the obstruction is almost always due to the development of a thrombus”<sup>13</sup> is not borne out by postmortem findings (Boyd,<sup>12</sup> Willius,<sup>13</sup> and Bedford<sup>14</sup>). The clinical use of the term “Coronary Occlusion, due to . . . (type of lesion)” is suggested.

In every case of sclerosis with or without thrombosis the occlusion was in the left coronary artery, and in only one case was it not in the anterior descending branch. The typical location for the largest atheromatous patch was from 2 to 3 cm. beyond the bifurcation of the main artery. In coronary disease the statistical evidence is in favor of a diagnosis of left coronary occlusion unless there is some definite evidence for right coronary occlusion, such as emboli in the lungs from a mural thrombus in the right ventricle. The right coronary artery was the seat of the lesion only in the case of embolism. The left coronary was the seat of the lesion in 16 of Wearn's 19 cases. Of 33 cases with autopsy, Willius<sup>13</sup> found the left coronary artery occluded in 29, the right in 2, and both in 2.

The areas of ischemic necrosis generally appeared in the anterior portion of the left ventricle near the apex; in four cases they also appeared in the left side of the interventricular septum. Although in 10 cases there were distinct gross acute infarcts, in only 3 of them were there conspicuous white infarcts. It is necessary to closely inspect the myocardium because “acute infarct formation may frequently be overlooked in the gross specimen.”<sup>14</sup> Ischemic necrosis was evident in every case microscopically. Such a heart embalmed immediately after death presents an easily discernible infarct which is characterized by a pink area of softness (devoid of arterial supply) surrounded by firm, fixed myocardium; postmortem blood clotting which has occurred before embalming may



obstruct a coronary artery and the surrounding myocardium may simulate an antemortem infarct, but then the microscopic picture of swollen muscle cells with granulation, loss of striation, shrunken nuclei, leucocytic infiltration and of a bordering zone of hyperemia is absent. An infarct which extends to the endocardium offers an opportunity for the formation of a mural thrombus (only one, Case 14, in this series) and resulting emboli, or, the infarct may extend to the pericardium and bring about a pericarditis. An infarct may become thin, form a myocardial aneurysm, and finally rupture. Myofibrosis, evidence of previous anemia, was found in all cases except the one of embolic occlusion. Generalized arteriosclerosis was found in 14 cases; and in 2 cases syphilis may have been the etiology of the sclerosis. An enlarged liver (1,750 gm. or over) occurred in only 50 per cent, a low figure. Albuminuria was present in 66 per cent of the cases in which urinalysis was done. Extracardial thrombi were found in only 2 cases. Meakins<sup>11</sup> in 62 cases of coronary artery thrombosis found extracardial thrombi in 40 cases.

TABLE III  
SUDDEN DEATH, ALL CAUSES

|              | CORO-<br>NARY<br>OC-<br>CLUSION | RUPTURED<br>AORTIC<br>ANEU-<br>RYSM | MYOCAR-<br>DITIS | PUL-<br>MONARY<br>EM-<br>BOLISM | UNDE-<br>TER-<br>MINED | HEAT<br>PROS-<br>TRATION | TOTALS |
|--------------|---------------------------------|-------------------------------------|------------------|---------------------------------|------------------------|--------------------------|--------|
| No. of Cases | 13                              | 4                                   | 3                | 2                               | 2                      | 1                        | 25     |
| Age 19       | 0                               | 0                                   | 0                | 0                               | 1                      | 0                        | 1      |
| 20-29        | 0                               | 1                                   | 0                | 0                               | 0                      | 0                        | 1      |
| 30-39        | 1                               | 0                                   | 1                | 0                               | 1                      | 0                        | 3      |
| 40-49        | 3                               | 1                                   | 2                | 0                               | 0                      | 1                        | 7      |
| 50-59        | 7                               | 1                                   | 0                | 2                               | 0                      | 0                        | 10     |
| 60-69        | 2                               | 0                                   | 0                | 0                               | 0                      | 0                        | 2      |
| 70-79        | 0                               | 1                                   | 0                | 0                               | 0                      | 0                        | 1      |
| Sex          |                                 |                                     |                  |                                 |                        |                          |        |
| Male         | 13                              | 4                                   | 3                | 0                               | 2                      | 1                        | 23     |
| Female       | 0                               | 0                                   | 0                | 2                               | 0                      | 0                        | 2      |
| Race         |                                 |                                     |                  |                                 |                        |                          |        |
| White        | 8                               | 2                                   | 1                | 0                               | 0                      | 0                        | 11     |
| Black        | 5                               | 2                                   | 2                | 2                               | 2                      | 1                        | 14     |

*Sudden Death.*—Excepting the accidental and traumatic deaths there were 25 sudden deaths from all causes. Table III shows the various causes with age, sex, and race figures. Cerebral hemorrhage is a clinical diagnosis commonly made at sudden death. Death from cerebral hemorrhage occurred in only 3 cases within twenty-four, twenty-three, and fourteen hours, respectively, after the strokes. "Of all sudden and unexpected deaths, apoplexy furnishes the fewest autopsies and inquests."<sup>15</sup> The largest single cause was coronary occlusion, which was more frequent than all other causes combined. It is interesting to note that of 11 cases of sudden death in the white race, 8 were of coronary occlusion. Table IV shows the various types of coronary artery occlusion which caused sudden death. Major McNabb,<sup>16</sup> in reviewing the 348 autopsies he performed in a year at the Canal Zone Board of Health Laboratory, found 14 cases of sudden death caused by coronary occlusion, and that "60 per cent of sudden deaths were a result of myocardial failure resulting from coronary lesions." In

a recent study of 198 patients brought in dead, Bedford<sup>14</sup> found "coronary artery disease, 80 cases, and atheroma of coronary arteries, 63 cases."

TABLE IV  
SUDDEN DEATH FROM CORONARY OCCLUSION

| No. of cases | SCLEROSIS | THROMBOSIS | SYPHILITIC<br>ATRESIA OF ORIFICE | TOTALS |
|--------------|-----------|------------|----------------------------------|--------|
|              | S         | 3          | 2                                | 13     |
| Age          |           |            |                                  |        |
| 30-39        | 0         | 0          | 1                                | 1      |
| 40-49        | 3         | 0          | 0                                | 3      |
| 50-59        | 5         | 1          | 1                                | 7      |
| 60-69        | 0         | 2          | 0                                | 2      |
| 70-79        | 0         | 0          | 0                                | 0      |
| Sex          |           |            |                                  |        |
| Male         | 8         | 3          | 2                                | 13     |
| Female       | 0         | 0          | 0                                | 0      |
| Race         |           |            |                                  |        |
| White        | 5         | 3          | 0                                | 8      |
| Black        | 3         | 0          | 2                                | 5      |

Scott<sup>17</sup> has aptly said "when a man beyond forty and in his usual health is seized with a severe attack of substernal pain, accompanied by shock, circulatory collapse, and dyspnea, we may assume that something definite has happened in the heart. If the patient dies in a few minutes, we may be reasonably sure that he has had a circulatory accident, most likely coronary. Indeed, more than 90 per cent of all individuals dying suddenly . . . will show at autopsy a cardiac lesion, coronary disease . . . or rupture of the heart at the site of an old or recent infarct. In a few cases a ruptured aorta is found. Very rarely does cerebral hemorrhage kill in a few minutes. In spite of these well-established facts we still find such erroneous diagnoses as 'acute indigestion,' or 'ptomaine poisoning' given as the cause of sudden death."

#### SUMMARY

In a study of 345 consecutive autopsies, of which 286 were upon bodies over one year of age, representing 208 negroes and 78 Caucasians, and which were performed one calendar year, there are presented: 18 cases of coronary occlusion produced by sclerosis without thrombosis (11), sclerosis with thrombosis (4), syphilitic constriction of a coronary artery orifice (2), and embolic occlusion (1); a discussion of the symptoms and pathology of coronary occlusion; and a discussion of 25 cases of sudden death, 13 of which were caused by coronary occlusion.

It is suggested that the inaccurate general term "coronary thrombosis" be used only when an actual thrombus is present.

The following *working rule* is suggested: *Unless there are signs definitely indicating other disease in a white male over forty years of age who dies within three hours after the onset of either respiratory, cardiac, or gastric symptoms, the cause of death is coronary occlusion.*

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## ALPHA DINITROPHENOL AND ITS INFLUENCE UPON METABOLISM\*

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MY INTEREST in this chemical substance was aroused during the Great War. The reports of cases of acute and others of chronic intoxication; the circulatory disturbances in the former and the progressive loss of flesh, anemia, and sweating in the latter, and even sudden death among some of the acute manifestations, naturally invited attention in relation to the manufacture of munitions, especially during that period when part of this industry devolved upon the United States. When the pernicious potentialities of certain of the chemicals utilized in the making of explosives were clearly recognized, successful methods of safeguarding those in the industry were introduced. It was this factor probably which caused a lag in the attention originally given to the subject. However, during the past two years especially, numerous articles have appeared, most of them in the French journals, particularly by pharmacologists and physiologists. Most of the published work has dealt with the effects of nitrated naphthols, specifically dinitro-alpha-naphthol, often referred to for the sake of brevity as D. A. N. Of the two isomers, alpha and beta-naphthol, the nitrated alpha-naphthol has received the greater attention. These two are derived from coal tar, or are prepared synthetically by hydrating the hydrocarbon naphthalene. The phenol group consists of nitrated hydroxyl derivatives of benzene. Both the naphthol and phenol groups are toxic in certain doses. Both are thermogenic, also in appropriate doses, but the naphthol group is more selective in its thermogenic properties, for certain animals fail to develop fever, irrespective of the route by which the drug is administered.

Renewed and widespread interest has been stimulated by the recent article by Cutting, Mehrtens and Tainter,<sup>1</sup> and still more recently by the publications of Anderson, Reed and Emerson<sup>2</sup> and by Tainter, Stockton and Cutting.<sup>3</sup> Each of these reports dealt with alpha-dinitrophenol (1:2:4), and, as the Preliminary Report of the Council<sup>4</sup> stated, "this compound is relatively new both to pharmacology and to clinical medicine." To those who know something of the literature of this subject, and particularly if they have had some clinical experience with this chemical, the advice of the Council will be regarded as sound. "There are limitations to and possible dangers from the use of the drug clinically. It should be used only under strictly controlled conditions." Pertinent to this quotation, it may be stated that the dose should be accurate, based upon the weight of the patient, and converting metric into apothecary's weight may introduce a possible error. I seriously question

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whether any prescription receives the calm and dispassionate attention it deserves, in the delicatessen and lunch counter drug store. Perhaps the abolition of the Eighteenth Amendment may aid in restoring that which has come to be spoken of as the older type of drug store.

In an editorial comment<sup>2</sup> on dinitrophenol, the writer raises the question as to whether there are cumulative effects from small doses repeated over long periods. To this it may be said that the drug has been given over a period of months with no appreciable ill effects. In doses over 10 mg. per kilo it will produce profuse, drenching sweats, fever, and very high metabolic rates. In the doses generally employed, 3 to 5 mg. per kilo, there is rarely even a slight elevation of temperature as measured by the clinical thermometer, no increase in heart rate, sweating is not always marked, but the metabolic rate begins to rise soon after the drug is taken, but falls to the patient's normal again in about forty-eight hours. The increased metabolic rate is not maintained after stopping the drug, even in those to whom it has been given over long periods of time. There is no proof therefore, based upon the specific action of the drug, that cumulative effects occur. Eruptions have been reported and edema, especially from the sodium salt which is much more soluble than the acid. Whether other idiosyncrasies exist, or whether it will manifest any ill effects in the presence of infections or disturbed metabolic states, cannot be stated definitely at this time. Both from animal experimental work, using nitrated naphthols, and from the clinical use of alpha dinitrophenol, it is fairly evident that these compounds should not be used in diabetes or in any phase of hyperthyroidism. Owing to the rapidity with which the average obese individual passes "from hand to mouth" so to speak, the difficulty in controlling diets, even in the hospital ward, in the absence of a well-organized metabolic department, and the almost uniform rapidity with which dinitrophenol speeds up metabolic rate, the chief interest seems to center in the possibility of weight reduction without detailed and specific supervision of the dietary. With this in view therefore, I solicited the cooperation of the metabolic department of the Philadelphia General Hospital, under the directorship of Dr. Edw. S. Dillon, Dr. W. G. Karr the metabolic chemist of the department, and Mr. Theodore V. Letonoff, assistant metabolic chemist.

The patient, J. S., a male, aged fifty-six, a chauffeur, was admitted to my ward on July 18, 1933. He complained of swelling and soreness of his ankles, of pain in various parts of his trunk and limbs, and that his excessive weight and dyspnea prevented him from working. Decidedly short, his average weight had been 168 to 170 pounds for some years. Nineteen months prior to his admission to hospital, he had reached 200 pounds. This remained fairly constant until seven months ago, when he began to gain more rapidly and went up to 245 pounds. Unable to work, probably economic conditions brought about a reduction in weight, for he was 214 pounds when first seen. He was not only obese, but he had in addition a nodular, circumscribed lipomatosis on limbs, thorax, and abdomen. As is not unusual, many of these areas were tender, he was subject to neuralgic pain, tired easily, a syndrome characteristic of adiposis

dolorosa and not unusual in the nodular, circumscribed variety. This nodular distribution he first noted thirty years ago and gradually the tumefactions increased in number, a few having become quite large. His bowels were regular, his habits good. He denied the use of alcohol. No syphilitic history, Kahn negative. Family history of no importance. His past history was good, but for a postnasal catarrh for some years and moderate deafness, especially in the right ear, which followed a severe cold ten years ago. For some months he had a productive cough and an occasional night sweat.

*Physical Examination.*—Not of pituitary type. No abnormal distribution of hair. Teeth in poor condition. A little pyorrhea. Tonsils enlarged. Few râles at bases of lungs. Heart: 13 cm. from midline to left border. No murmur. Muscle tone suppressed and distant. Pulse rate 80. Blood pressure, 142 systolic; 90 diastolic. Abdomen: No gross pathology noted. Genitalia: normal. Eyes: Pupils reacted normally to light. Veins overfull. No pathologic findings on examination of fields. Skull: X-ray of bones of skull was negative. No evidence of intracranial pressure. Sella slightly larger in vertical diameter than normal. A narrow bridge of bone passed from the anterior to the posterior clinoid, but the clinoids were otherwise normal. One of the circumscribed, fatty masses was removed for a biopsy. Dr. R. P. Custer reported the finding of typical adult fat supported by the usual fibrous trabeculae.

Before any of the drug was given to him, he had a basal metabolism rate of plus 6 on one occasion, plus 8 on another. His blood counts and urine were normal. Blood cholesterol 270, and his calcium 12.6 and 10.3 at different times. Liver function by the bromsulphaline and by the Quick, benzoic acid methods were both normal. Without dietary restriction, limiting fluid to 1,500 c.c. daily, he lost four pounds in nine days, his weight now being 210 pounds on July 27, 1933. On Aug. 9, 1933, he was placed upon a diet of

| Proteins | Fats | Carbohydrates | Calories |
|----------|------|---------------|----------|
| 100      | 50   | 100           | 1,250    |

Fifteen days later, on Aug. 24, 1933, he weighed 205 pounds, a total loss of but 9 pounds in thirty-seven days, approximately  $\frac{1}{4}$  of a pound a day. On Sept. 18, 1933, he was admitted to the men's metabolic ward and the metabolic study was begun. At this time he weighed 197 pounds, a total loss of 17 pounds in sixty-two days, a little over  $\frac{1}{3}$  of a pound a day since his admission to the hospital. Up to this date he had received no dinitrophenol and during this period his basal metabolism rate was plus 6 on July 27, 1933, and plus 8 on Aug. 24, 1933. On Sept. 18, 1933, his fasting blood sugar was 100; Urea N. 0.013; CO<sub>2</sub>, 59 vol. per cent. For the next three days he was kept upon the following diet:

| Proteins | Fats | Carbohydrates | Calories |
|----------|------|---------------|----------|
| 100      | 50   | 100           | 1,250    |

Sept. 22, 1933, his diet was changed as follows:

|          |      |               |          |
|----------|------|---------------|----------|
| Proteins | Fats | Carbohydrates | Calories |
| 85       | 60   | 200           | 1,680    |

## Sugar Tolerance

|                    |         |
|--------------------|---------|
| Fasting            | 83 mg.  |
| $\frac{1}{2}$ hour | 132 mg. |
| 1 hour             | 134 mg. |
| 2 hours            | 90 mg.  |
| 3 hours            | 61 mg.  |

| TIME           | B.S.<br>MG.                                   | URINE<br>GM. N.<br>PER HR.                  | R.Q. | B.M.R. | CAL. PER HR. BURNED FROM |     |       |        |
|----------------|-----------------------------------------------|---------------------------------------------|------|--------|--------------------------|-----|-------|--------|
|                |                                               |                                             |      |        | PROT.                    | FAT | CARB. | WEIGHT |
| Sept. 25, 1933 | No breakfast or lunch, regular supper         |                                             |      |        |                          |     |       |        |
| A.M. 7:30      | 92                                            | 0.511                                       | 0.78 | + 6    | 13                       | 49  | 14    |        |
| 6 A.M.-9 A.M.  |                                               |                                             |      |        |                          |     |       |        |
| 8:50           | 0.45 gm. dinitrophenol                        |                                             |      |        |                          |     |       |        |
| 9:15           |                                               | 93                                          | 0.75 | +37    | 11                       | 87  | 12    |        |
| 11:15          |                                               |                                             | 0.74 | +37    |                          |     |       |        |
| 9 A.M.-12 Noon |                                               | 0.415                                       |      |        | 11                       | 87  | 6     |        |
| P.M. 1:15      |                                               | 0.424                                       |      |        |                          |     |       |        |
| 12 Noon-3 P.M. |                                               |                                             |      |        |                          |     |       |        |
| 3:15           | 102                                           |                                             | 0.74 | +44    | 11                       | 92  | 8     |        |
| Sept. 26, 1933 | No breakfast                                  |                                             |      |        |                          |     |       |        |
| A.M. 8:20      |                                               | 0.447                                       | 0.73 | +24    | 12                       | 71  | 6     |        |
| 6 A.M.-9 A.M.  | 0.45 gm. dinitrophenol                        |                                             |      |        |                          |     |       |        |
| 9:00           |                                               |                                             | 0.73 | +52    | 13                       | 89  | 7     |        |
| 11:00          |                                               |                                             |      |        |                          |     |       |        |
| 9 A.M.-12 Noon |                                               | 0.492                                       |      |        |                          |     |       |        |
| P.M. 1:00      | Regular lunch and supper                      |                                             | 0.69 | +54    | 13                       | 98  | 0     |        |
| Sept. 27, 1933 |                                               |                                             | 0.73 | +27    |                          |     |       |        |
| A.M. 8:50      | Regular breakfast plus 0.36 gm. dinitrophenol |                                             |      |        |                          |     |       |        |
| 9:00           | Regular lunch                                 |                                             | 0.85 | +72    |                          |     |       |        |
| 11:00          | and supper                                    |                                             |      |        |                          |     |       |        |
| Sept. 28, 1933 |                                               |                                             | 0.80 | +24    | 13                       | 51  | 25    | 194    |
| A.M. 8:50      | 101                                           | 100 gm. glucose plus 0.45 gm. dinitrophenol |      |        |                          |     |       |        |
| 9:25           |                                               |                                             | 0.81 | +38    | 13                       | 55  | 31    |        |
| 10:25          | 180                                           |                                             | 0.93 | +41    | 13                       | 12  | 76    |        |
| 11:25          | 118                                           |                                             | 0.91 | +33    | 13                       | 20  | 62    |        |
| 12:25          | 74                                            |                                             |      |        |                          |     |       |        |
| 9:25-12:25     |                                               | 0.491                                       |      |        |                          |     |       |        |
| Oct. 1, 1933   | Readmitted to Men's medical ward              |                                             |      |        |                          |     |       |        |
| Oct. 3, 1933   |                                               |                                             | 0.76 | + 7    |                          |     |       | 193    |
| A.M. 8:45      |                                               |                                             |      | +36    |                          |     |       |        |
| Oct. 7, 1933   |                                               |                                             |      |        |                          |     |       |        |

It will be noted that from Sept. 18 to Oct. 3, 1933, a total of fifteen days, he lost an additional four pounds in weight, or approximately one-quarter pound per day. Following his return to the medical ward, he was not given dinitrophenol for a couple of days, hence his return to a basal metabolic rate of plus 7, with a respiratory quotient of 0.76. The dose of 0.45 gm. given at one time on September 25 and 26, the first two days of the observation, was equivalent to 5 mg. per kilo. The same dose was given on September 28, but on September 27 the dose was less, being but 4 mg. per kilo. Despite the smaller dose on this day, the basal metabolism rate rose to plus

72, very much higher than it had done on September 25 and 26, which were fasting periods. We interpreted this as being due to the specific dynamic action of the food, plus the elevation of rate due to the drug. It will be evident, therefore, that in the ambulatory individual on a full diet, even smaller doses should be employed. One woman who was on a full diet and ambulatory, except during the period of study of her rates, with a basal rate of minus 27, was given but 1 mg. per kilo. One hour later her rate was minus 22, and after another hour it was minus 13. In none of the various observations above cited was there any appreciable change in temperature and pulse or respiration. On another occasion, I intend to present other phases of this subject, but at this moment my chief concern is to advocate a small dose once a day of 3 mg. per kilo, or even better, but 2 mg. per kilo over a long period of time, to the obese individual who is not a diabetic, and is engaged in a daily routine, i.e., not bedridden. This I regard as perfectly safe. Weight will not be lost rapidly and many days may elapse after the drug is first given before the weight begins to fall, but it is fairly steady and continuous subsequently. In the case of J. S., the subject of this special study, as previously stated, there was a loss of but 17 pounds in sixty-two days before dinitrophenol was given. Under a strictly controlled diet, three partial fasting days being included in a total of seventeen days up to the day of his discharge from the hospital on October 12, his weight fell to 187 pounds, a further loss of 10 pounds, or at the rate of three-fifths of a pound per day. The metabolic study in the case of J. S. shows the time factor in the development and the extent to which the basal metabolism rate is raised on a dose of but 5 mg. per kilo. At the same time the respiratory quotient was slightly lowered on September 25. With but one meal on this day and no breakfast next morning, a further lowering of the respiratory quotient is what would be expected. This progressive drop in the respiratory quotient, during the first two days of the study, does not necessarily mean that the oxidation of fat is stimulated, though very suggestive. The low calory intake must also be considered. It seems equally evident that the extra oxidation caused by the drug was not accomplished at the expense of protein or carbohydrate. The progressive increase in calories of fat burned per hour on each of the first two days, when considered in the light of the other findings portrayed, makes it very probable that the very evident increase in oxidation is at the expense of the fat. A side light is shed on this feature in our obese cases of cardiac decompensation. Presumably the interface phenomena of fat and water in the edematous cardiac case, in some way affords a firmer binding of the two. The basal metabolic rate does not mount quite so high, and until the tissue fluids have been mobilized and in large part excreted, there is no appreciable loss of flesh and the loss is very gradual when it does occur. An attempt to reduce the weight of the obese individual with myocardial involvement is manifestly justifiable, but it remains in doubt as to whether it is advisable to attempt it by means of dinitrophenol.

The study of sugar tolerance after 100 gm. of glucose plus 5 mg. per kilo of the drug, indicates that sugar utilization during the first hour may be inhibited. At the end of the second hour the blood sugar is essentially normal,



and the respiratory quotient of 0.93 is what one would anticipate, even had no drug been given. In experimental work on animals with dinitro-alpha-naphthol, an early rise in the blood sugar has been noted and the liver and muscle glycogen are reduced.

It is of further interest to note that a single dose of 5 mg. per kilo, given during the fasting state, caused a rise in the rate of 31 per cent in less than two and one-half hours, and a total rise of 38 per cent in about six and one-half hours. Twenty-four hours after the administration of the drug, the rate remained 18 per cent above his basal rate of the previous day. On another occasion, two hours after a dose one mg. less per kilo, given with his regular breakfast, there was a rise of 45 per cent in his rate, but this was regarded as a summation effect. The rapid action of the drug upon heat production in the fasting state was very similar on the two days. On the first day this was 31 per cent and on the second day 28 per cent, each in a little over two hours. Oxidation was much less rapid and less pronounced when the drug was given with a glucose meal, than when it was given alone and in the fasting state.

#### COMMENT

1. A metabolic study of the effects of dinitrophenol has been reported.
2. The rapid increase and degree of oxidation have been shown, (a) in the fasting condition, (b) when given with a glucose meal, (c) the summation effect of drug and regular meal.
3. The probability that oxidation occurs at the expense of fats has been suggested.
4. The question of dosage has been considered and in the light of present knowledge, doses of 2 to 3 mg. per kilo at one dose daily have been advised.
5. It would seem unwise to use it in a case of diabetes or in an edematous individual.
6. In appropriate doses, though its oxidative properties are striking, weight loss is not rapid, nor is there any appreciable effect upon temperature, pulse, or respiration.

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## ABSORPTION OF DEXTROSE FROM THE HUMAN GASTROINTESTINAL TRACT\*

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IN 1925, Cori,<sup>1</sup> as a result of an ingenious method by which absorption of saccharides from the entire gastrointestinal tract of rats was studied, came to the conclusion that (1) a proportionality exists between the absorbing surface of intestine and body weight in growing male and female rats, and (2) the rate of absorption of hexoses is constant and is independent of the absolute amount and of the concentration of sugar present in the intestine. He calculated the absorption rate to be 1.78 grams of dextrose per kilogram of rat.

Trimble, Carey and Maddock,<sup>2</sup> utilizing Cori's technic in dogs, made substantially the same observations. They found that the rate of absorption of dextrose from the intestinal tract of dogs is one gram per kilogram of body weight per hour; also, that this rate is constant and not influenced by: (a) the concentrations; (b) the absolute weight of dextrose ingested; (c) lengths of absorption period; (d) weights of the animals used; and, (e) excitement occasioned by experimentation.

MacKay and Bergman,<sup>3</sup> also employing Cori's method of study, made findings entirely at variance with those of the latter. They concluded that (1) the absorption rate of dextrose per unit of time from the intestinal tract of male and female rats of various ages bears a more constant relation to body surface than to body weight, and (2) the absorption coefficient (amount of dextrose absorbed per unit of body surface per hour) is raised by increases in either the amount or concentration of dextrose administered. Whatever the dose, the rate of absorption decreases with time after it is given. Pierce, Osgood and Polansky<sup>4</sup> and Burget, Moore and Lloyd<sup>5</sup> previously had made observations which are in agreement with those of MacKay and Bergman. In addition they found that the total amount of dextrose absorbed and the amount absorbed per 100 gram of body weight per hour varies within wide limits. These variations are not dependent on body weight or upon the actual amount of dextrose fed.

In animal experiments, then, the findings and conclusions of the various investigations are diametrically opposed to each other.

Jacobsen<sup>6</sup> found that the administration of water does not influence the "fasting" blood sugar level. Strouse<sup>7</sup> made similar observations, and in

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addition noted that the "fasting" blood sugar level is not changed by increased excretion of water. Boe<sup>3</sup> found in dogs that the administration of a dilute solution of dextrose causes only an insignificant rise in blood sugar; whereas a more concentrated solution caused a much higher rise. This may be taken as evidence that the more concentrated the solution the greater is the rate of absorption.

Direct methods of study of the absorption of dextrose from the gastrointestinal tract are, of course, not applicable to man. We are, as a consequence, deplorably ignorant of this subject as far as human beings are concerned. From a study of the respiratory quotient curve after the administration of dextrose, Johansson<sup>9</sup> concluded that the maximum rate of absorption of dextrose in man is between 80 and 100 gm. per hour.

In this paper will be reported the results of an investigation, by an indirect method, of the laws governing the absorption of dextrose from the human gastrointestinal tract.

In a normal individual it is practically impossible to raise the blood sugar level above 180 mg. per 100 c.c. of venous blood on the administration of dextrose by mouth, for the amount of insulin secreted is easily capable of causing the abstraction of dextrose from the circulation regardless of the rapidity with which it is absorbed from the intestine. In diabetes mellitus, however, hypoinsulinism exists and the amount of insulin secreted at any particular time is fixed and dependent on the severity of the case. The amount of dextrose that the individual diabetic can metabolize per unit time follows the equation determined by Allan.<sup>8, 10</sup> If the absorption of a certain amount of dextrose per unit time in a given diabetic gives rise to a certain blood sugar curve, the absorption of a greater amount will cause a higher blood sugar curve because the amount of insulin secreted remains constant. In the same diabetic, the greater the amount of dextrose absorbed in a given time, the higher will the blood sugar curve be. If, then, one gives to the same diabetic varying amounts of dextrose, two days apart, the blood sugar curves will be equal in height or higher with the larger dose, depending on whether the rate of absorption is the same or greater with the larger dose.†

#### METHOD OF PROCEDURE

With this a basis, experiments were performed on 14 female diabetics. To each one two doses of dextrose were given; a large one on one day, and a smaller one two days later. To a group of three, 125 gm. of dextrose were given on the first day and 25 gm. two days later; to a second group of six,

\*The dextrose equivalent of insulin in any given case is not a constant, but is represented by the equation  $g(u)^{0.33} = 10^{1.66}$  where  $g$  is the amount of dextrose metabolized by each unit, and  $u$  is the number of units of insulin given. The dextrose equivalent of insulin is increased if the amount of insulin remains constant and the amount of dextrose increased, or if the amount of dextrose remains constant and the amount of insulin decreased; the dextrose equivalent is decreased if the amount of insulin is increased and the amount of dextrose remains constant, or if the amount of dextrose is decreased and the amount of insulin remains constant. In this situation the amount of insulin remains constant. If more dextrose is absorbed the dextrose equivalent will be increased, so that the rate of rise of the blood sugar curve will be less and less with increasing rate of absorption of dextrose.

†It is realized that the blood sugar level is influenced by the secretions of the thyroid, adrenal and pituitary glands, and other factors. It is fair to assume that these factors, like insulin, remain constants during the period of experimentation.

either 100 or 150 gm. were given on the first day and 50 gm. two days later; to a third group of five, 150 gm. were given on the first day and 75 gm. two days later. The anhydrous dextrose was dissolved in distilled water and made up to 500 c.c. An hour after the administration of the dextrose 250 c.c. of water were given. The total volume was therefore 750 c.c. in each case for both the large and the small dose.

The dextrose was given in the morning after a fast of fourteen hours. Blood (venous) specimens were taken at the "fasting level," immediately before ingestion, and forty-five minutes, two hours, and in some cases, three hours after the ingestion of the dextrose. The sugar content of the blood was determined by the macro Folin-Wu method. The urine was collected at the end of the first, second, and in some of the cases, third hour after ingestion of the dextrose, and its sugar content determined by Benedict's method.

#### ANALYSIS OF RESULTS

The results are incorporated in Table I. After careful consideration it was decided that the best method of determining the influence of the different doses on the blood sugar curve was to calculate the differences in rise of blood sugar concentration when the large dose and the small one were given, from the "fasting level" to the "forty-five-minute," "two-hour," and "three-hour" levels, respectively. To illustrate: in Case 1 (Table I) the "fasting level" when 125 gm. of dextrose were given was 214, and the "forty-five-minute level," 332, indicating a rise in blood sugar concentration of 118 mg. per 100 c.c. of blood. When 25 gm. of dextrose were given the "fasting level" was 230 and the "forty-five-minute level," 332, indicating a rise in blood sugar concentration of 102 mg. per 100 c.c. of blood. The difference in elevations is 16. A plus sign is arbitrarily placed in front of the 16 to indicate that the rise is greater when the larger dose is given. A minus sign, on the other hand, indicates that the rise is greater when the smaller dose is given.

Analyzing the blood sugar concentrations at the "two-hour level" it will be observed that in Group I, in all cases, the rise is markedly greater with the larger dose. This also holds true for all the cases in Group II, although the difference is much less and in one case (Case 9) is within the limit of error of the method used to analyze the blood. In Group III, however, a striking change has taken place. Here, in four out of five cases, the rise is greater when the smaller dose is given. This is of fundamental significance for it indicates that at the end of two hours at least as much dextrose is being absorbed when 75 gm. are given as when 150 gm. are given; in other words, *the rate of absorption of dextrose is the same regardless of the absolute amount in the intestine.* As a corollary to this it may be stated that *the rate of absorption is constant.*

An interpretation may now be given for the greater rise in blood sugar concentration at the "two-hour level" in Groups I and II when the larger dose is given. The reason is that the dextrose, when the smaller dose is given, has been completely absorbed before the end of two hours so that the blood sugar concentration has been dropping for some time before the "two-hour

level" is reached. This is brought out in Group II at the "three-hour level." Here in every one of the four cases studied, the difference in rise is decidedly greater than at the "two-hour level." Also, in Group I, the difference in rise at the "two-hour level" is much greater than in Group II because the blood sugar concentration in the former has been dropping for a much longer time.

It follows, then, that when 75 gm. of dextrose are given (Group III), the amount remaining in the gastrointestinal tract at the end of two hours is less than 25 gm.; whereas when 150 gm. are given, the amount remaining at the end of two hours is between 75 and 100 gm. The important deduction from these findings is *that the amount of dextrose absorbed in two hours from the gastrointestinal tract of the average adult diabetic lies somewhere between 50 and 75 gm.* Since there is no reason to suppose that the rate of absorption differs in normals from diabetics, this statements holds for all adults.

Analyzing the "forty-five-minute level" one finds that in 13 out of the 14 cases the rise is greater with the larger dose. In 6 out of the 14 cases the figure for the difference in rise is within the limit of error of the method used for analyzing the blood. In spite of this, one is impressed that the difference in rise is somewhat greater when the larger dose is given. It is no greater in the group receiving 25 gm. than in the one receiving 75 gm. What is the cause for this greater rise with the larger dose at the "forty-five-minute level"?

It was stated above that some investigators<sup>3, 4, 5</sup> found that the rate of absorption is increased by increase in concentration of dextrose administered; also, that Boe<sup>8</sup> observed that the level of the blood sugar curve is higher when a more concentrated solution is given. In the experiments recorded in Table I, the volume was kept constant; the concentration necessarily varied.

The question then is: Is the greater rise with the larger dose due to the fact that the solution administered was more concentrated? To determine this point, the following experiment was performed. To seven patients, suffering from diabetes mellitus, varying doses of dextrose, of equal concentration, namely 32 per cent, were given two days apart. On one day 125 gm. and two days later 45 gm. of dextrose were administered. Venous blood was taken at the "fasting level" immediately before ingestion, and forty-five minutes after ingestion. The results are incorporated in Table II. It is to be observed that in 6 out of 7 cases the rise is greater with the larger dose, and that the differences in rise are of the same magnitude as in Table I. The conclusion to be drawn is that the height of the blood sugar curve is not influenced by differences in concentration of the dextrose administered. As a corollary it may be stated that the rate of absorption of dextrose is not influenced by this factor. I am at a loss to explain the greater rise in blood sugar concentration with the larger dose at the "forty-five-minute level."

The observations made above have a practical value. The dextrose tolerance test, especially when combined with a simultaneous study of the respiratory quotient curve,<sup>11</sup> constitutes the best method of determining the presence of diabetes mellitus and also its severity.<sup>12</sup> In a symposium on the

dextrose tolerance test, Gray<sup>13</sup> lists the test substances and doses used by various investigators in the study of carbohydrate metabolism. They are as follows: from 20 to 25 gm. of dextrose; 50 gm. of dextrose; 70 gm. of dextrose; 100 gm. of dextrose; from 150 to 200 gm. of dextrose; 100 gm. of levulose; from 50 to 100 gm. of cane sugar; 25 gm. of starch; 50 gm. of starch; from 70 to 100 gm. of starch; and a mixed meal. In addition it may be mentioned that Janney and Isaacson<sup>14</sup> use 1.75 gm. of dextrose per kilogram of body weight. The choice of these doses and substances in all instances is purely arbitrary.

John<sup>15</sup> performed dextrose tolerance tests on 50 patients with diabetes, using 100 gm. of dextrose as a test dose. He found that in 86 per cent of the cases the maximum rise occurs within two hours after the ingestion of the dextrose. This also holds true when 150 gm. of dextrose are given, for in 5 cases in which this dose was given by me (see Table I) all had the maximum rise within the first two hours. The character of the curve *after*

TABLE I

REPEATED DEXTROSE TOLERANCE TESTS IN DIABETICS USING VARYING AMOUNTS OF DEXTROSE  
(VOLUME OF FLUID CONSTANT)

|           | CASE NO. | DOSE OF DEXTROSE<br>IN GRAMS | CONCENTRATION OF SUGAR IN BLOOD* |                                    |                       |                         |                       |                           |                       | EXCRETION OF DEXTROSE IN<br>URINE AFTER INGESTION IN<br>GRAMS |                |               |
|-----------|----------|------------------------------|----------------------------------|------------------------------------|-----------------------|-------------------------|-----------------------|---------------------------|-----------------------|---------------------------------------------------------------|----------------|---------------|
|           |          |                              | FASTING<br>LEVEL                 | FORTY-FIVE MIN-<br>UTES POST CIBUM | DIFFERENCE IN<br>RISE | TWO HOURS<br>POST CIBUM | DIFFERENCE IN<br>RISE | THREE HOURS<br>POST CIBUM | DIFFERENCE IN<br>RISE | FIRST<br>HOUR                                                 | SECOND<br>HOUR | THIRD<br>HOUR |
|           |          |                              |                                  |                                    |                       |                         |                       |                           |                       |                                                               |                |               |
| GROUP I   | 1        | 125                          | 214                              | 332                                | +16                   | 500                     | +216                  |                           |                       | 0.38                                                          | 3.57           |               |
|           |          | 25                           | 230                              | 332                                |                       | 300                     |                       |                           |                       | 0.14                                                          | 0.77           |               |
|           | 2        | 125                          | 150                              | 250                                | +33                   | 300                     | + 83                  |                           |                       | 0.35                                                          | 4.17           |               |
|           |          | 25                           | 187                              | 250                                |                       | 250                     |                       |                           |                       | 0.20                                                          | 0.24           |               |
|           | 3        | 125                          | 230                              | 374                                | +70                   | 460                     | +230                  |                           |                       | 2.14                                                          | 3.25           |               |
|           |          | 25                           | 300                              | 374                                |                       | 300                     |                       |                           |                       | 1.35                                                          | 0.43           |               |
| GROUP II  | 4        | 100                          | 214                              | 374                                | + 8                   | 460                     | +144                  |                           |                       | 0.62                                                          | 6.34           | 8.31          |
|           |          | 50                           | 258                              | 410                                |                       | 360                     |                       |                           |                       | 2.0                                                           | 7.96           | 6.00          |
|           | 5        | 100                          | 300                              | 416                                | +20                   | 500                     | +104                  | 428                       | +134                  | 8.73                                                          | 16.30          | 7.80          |
|           |          | 50                           | 278                              | 374                                |                       | 374                     |                       | 272                       |                       | 5.25                                                          | 8.48           | 3.50          |
|           | 6        | 100                          | 230                              | 322                                | - 4                   | 360                     | + 34                  | 372                       | +110                  | 2.10                                                          | 9.90           | 4.24          |
|           |          | 50                           | 278                              | 374                                |                       | 374                     |                       | 260                       |                       | 2.57                                                          | 5.30           | 1.28          |
|           | 7        | 100                          | 375                              | 554                                | + 8                   | 600                     | + 54                  | 556                       | +128                  | 7.00                                                          | 18.50          | 12.80         |
|           |          | 50                           | 375                              | 546                                |                       | 546                     |                       | 428                       |                       | 7.26                                                          | 19.33          | 2.80          |
|           | 8        | 150                          | 263                              | 468                                | +71                   | 536                     | +157                  | 500                       | +209                  | 2.68                                                          | 11.80          | 12.3          |
|           |          | 50                           | 312                              | 446                                |                       | 428                     |                       | 340                       |                       | 3.50                                                          | 7.90           | 2.62          |
|           | 9        | 150                          | 192                              | 332                                | +12                   | 332                     | + 12                  | 262                       |                       | 1.80                                                          | 5.90           | 4.00          |
|           |          | 50                           | 172                              | 300                                |                       | 300                     |                       |                           |                       | 1.20                                                          | 3.90           |               |
| GROUP III | 10       | 150                          | 246                              | 428                                | +36                   | 428                     | -106                  |                           |                       | 0.60                                                          | 1.20           |               |
|           |          | 75                           | 258                              | 404                                |                       | 546                     |                       |                           |                       | 1.20                                                          | 3.60           |               |
|           | 11       | 150                          | 227                              | 400                                | +13                   | 484                     | + 43                  | 374                       |                       | 2.00                                                          | 5.66           | 7.70          |
|           |          | 75                           | 214                              | 374                                |                       | 428                     |                       |                           |                       | 0.90                                                          | 4.00           |               |
|           | 12       | 150                          | 166                              | 300                                | +34                   | 300                     | - 68                  | 240                       |                       | 1.42                                                          | 4.84           |               |
|           |          | 75                           | 172                              | 272                                |                       | 374                     |                       |                           |                       | 0.60                                                          | 1.00           |               |
|           | 13       | 150                          | 192                              | 352                                | +54                   | 322                     | - 40                  | 250                       |                       | 3.50                                                          | 8.90           | 4.75          |
|           |          | 75                           | 178                              | 284                                |                       | 348                     |                       |                           |                       |                                                               |                |               |
|           | 14       | 150                          | 333                              | 546                                | +45                   | 600                     | - 33                  |                           |                       | 3.94                                                          | 10.50          |               |
|           |          | 75                           | 300                              | 468                                |                       | 600                     |                       |                           |                       | 3.25                                                          | 7.10           |               |

\*In milligrams per 100 c.c. of blood.

the first two hours is determined by the size of the dose; the larger the dose, the longer it will take for the "fasting level" to be attained. In performing a dextrose tolerance test it is of value not only to determine whether the blood sugar curve is or is not higher than normal but also the maximum rise obtainable. From Table I it can be seen that 75 gm. of dextrose is the smallest dose that will give a maximum rise in blood sugar in the average adult diabetic.

It is also important to note that a dose of 50 gm. of dextrose or less may fail to disclose a potential or very mild diabetic if the "fasting level" is normal. The reason is that because of the complete absorption of the dextrose before the end of two hours post cibum, the blood sugar may drop to the "fasting level" at the end of the two hours. This event would not occur if 75 gm. of dextrose are given. Seventy-five grams of dextrose is the minimum dose that can be used to detect diabetes mellitus. It is accordingly proposed that 75 gm. of dextrose be used as the test dose.

A point which is not entirely relevant to the subject of this paper may, however, with value be discussed here. Does the administration of large quantities of dextrose, such as the patients received in these experiments, lead to a breaking down of tolerance? Joslin<sup>16</sup> strongly deprecates the use of the dextrose tolerance tests in diabetics, although he has never seen one injured by them. Ohler<sup>17</sup> also argues that on theoretical grounds the giving of large quantities of dextrose is harmful. Allen,<sup>18</sup> in his work on partially depancreatized dogs, found that excessive feeding of carbohydrates, especially where given in a rapidly absorptive form, such as dextrose, leads to a rapid breaking down of tolerance. Several years ago I<sup>12</sup> observed for several months seven diabetics who had had two dextrose tolerance tests performed two days apart. The test dose was 100 gm. of dextrose. None showed the slightest change in tolerance as a result of this procedure. Nor was there any depreciation of tolerance in any of ten patients of Table I as a result of receiving

TABLE II

REPEATED DEXTROSE TOLERANCE TESTS IN DIABETICS USING VARYING AMOUNTS OF DEXTROSE  
(CONCENTRATION OF SOLUTION CONSTANT)

| CASE | DOSE<br>OF DEXTROSE<br>IN GRAMS | CONCENTRATION OF SUGAR IN BLOOD* |                                    |                       |
|------|---------------------------------|----------------------------------|------------------------------------|-----------------------|
|      |                                 | FASTING<br>LEVEL                 | FORTY-FIVE MIN-<br>UTES POST CIBUM | DIFFERENCE IN<br>RISE |
| 1    | 125                             | 258                              | 428                                | +19                   |
|      | 45                              | 223                              | 374                                |                       |
| 2    | 125                             | 214                              | 374                                | +22                   |
|      | 45                              | 246                              | 374                                |                       |
| 3    | 125                             | 142                              | 200                                | +12                   |
|      | 45                              | 130                              | 176                                |                       |
| 4    | 125                             | 131                              | 196                                | +10                   |
|      | 45                              | 107                              | 162                                |                       |
| 5    | 125                             | 283                              | 428                                | +29                   |
|      | 45                              | 258                              | 374                                |                       |
| 6    | 125                             | 227                              | 374                                | +52                   |
|      | 45                              | 205                              | 300                                |                       |
| 7    | 125                             | 250                              | 384                                | - 4                   |
|      | 45                              | 238                              | 376                                |                       |

\*In milligrams per 100 c.c. of blood.

the large quantities of dextrose. It may be stated categorically that the giving of large quantities of dextrose does not cause the slightest depreciation of carbohydrate tolerance in diabetics.

#### SUMMARY AND CONCLUSIONS

The absorption of dextrose from the gastrointestinal tract has been studied quite intensively in animals. The results and conclusions of the various investigators are conflicting.

This subject has been almost completely neglected in man.

An indirect method of studying this subject in human beings is described. It consists in performing repeated dextrose tolerance tests on diabetics, using varying doses of dextrose. The rationale for this procedure is outlined.

The conclusions drawn from the data obtained are as follows:

1. The rate of absorption of dextrose from the human gastrointestinal tract is the same regardless of the absolute amount present. A corollary to this is that the rate of absorption of dextrose is constant.
2. The amount of dextrose absorbed from the gastrointestinal tract of the average adult in two hours lies somewhere between 50 and 75 gm.
3. The rate of absorption from the gastrointestinal tract is not influenced by the concentration of the solution of dextrose administered.
4. Seventy-five grams of dextrose is proposed as a standard dose to be used in performing a dextrose tolerance test.
5. The ingestion of large quantities of dextrose by patients with diabetes does not cause the slightest depreciation of their tolerance for carbohydrates.

NOTE: I wish to express my thanks to Miss Louise Zelenko for technical assistance.

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## HISTOCHEMICAL STUDIES OF ORGANS OF TUMOR-BEARING RATS BY THE MICROINCINERATION METHOD<sup>2</sup>

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THE study of the amount, character and distribution of mineral matter in normal and malignant tissues has been recently advanced by the introduction of the microincineration method (Policard,<sup>1</sup> Schultz-Brauns,<sup>2</sup> Scott and Horning<sup>3</sup> and others). The value of the observations made, especially those of comparative character, is, however, impaired by the fact, that the great majority of the investigators used paraffin sections in their work.

Fixation and dehydration of tissues cause not inconsiderable changes in the amount and distribution of the mineral substances in the cells (Schultz-Brauns,<sup>2</sup> Policard and Okkels,<sup>4</sup> Tschopp,<sup>5</sup> Cowdry<sup>6</sup>). The degree and type of changes produced by fixating and dehydrating agents depend upon their chemical nature, a time factor and possibly also on the physicochemical constitution of the tissues.

Many of these defects in the proper preparation of sections for microincineration have been eliminated in the technic of frozen sectioning developed by Schultz-Brauns,<sup>2</sup> who modified the commonly used method by cutting unfixed tissue with a knife cooled with carbon dioxide to  $-15^{\circ}$  to  $20^{\circ}$  C. Losses of mineral substances are thereby completely avoided and the possibility of artificial displacements of the cellular mineral skeleton is markedly decreased.

The present communication represents a report of the results of an investigation on the mineral skeleton of malignant cells studied in frozen sections of fresh, unfixed tissue cut according to a modification of the method of Schultz-Brauns (Hueper<sup>7</sup>). In the second part of the paper observations are recorded which were made on normal and neoplastic tissues of rats treated with parathormone and viosterol.

### PART I. MINERAL STRUCTURE OF MALIGNANT TISSUE

The tumor tissue used was obtained from rats which had been inoculated with a transplantable sarcoma, Philadelphia No. 1 and a transplantable medullary carcinoma, Walker No. 256 respectively, a detailed histologic description of which has been published in a previous paper (Waldschmidt-Leitz, McDonald, Hueper and Coworkers<sup>8</sup>). Sections were prepared with the cooled

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knife technic from fresh, unfixed tumor tissue removed from the peripheral parts of the tumors. Alternating sections were stained and incinerated respectively. For the incineration an electrically heated quartz furnace was used, the maximum temperature of which was adjusted to 600° C. The sections were kept in the oven for about forty-five minutes. They were left in the incinerator until they were cool and were then covered with a glass coverslip which was sealed with paraffin. A Zeiss cardioid condenser was used for the dark-field examination. A small Leitz camera which had no bellows and which could be attached to the eyepiece was used in the photographic work. The sections to be stained were first put into 95 per cent alcohol and then stained in the ordinary way with hematoxylin-eosin or hematoxylin-phloxin.

The stained sections show more or less densely packed tumor cells, embedded in a loose connective tissue stroma. The nuclei are relatively large and contain dark stained chromatin material arranged in irregular lobules and threads. A complete or incomplete ringlike accumulation of the chromatin in the periphery of the nuclei is exceptional. The cytoplasm is moderate to scanty in amount and is indistinctly outlined in the sarcomatous tumor, while rather well defined in the carcinomatous neoplasm. Small localized foci of degeneration and necrosis are scattered in moderate number in the tissue. In these degenerative areas pyknotic nuclei are numerous. Pseudopapillary-cystic formations are seen in some of the regressive foci. The necrotic areas are pink in color and contain chromatic nuclear fragments. Diffuse, usually perivascular hemorrhages are occasionally observed.

The ash pictures of the tumor sections vary considerably in different portions of the same section. In the areas corresponding to the above described healthy parts of the tumor a scanty bluish to bluish gray reflecting matrix of very small granules is present. Embedded in it numerous, smaller and larger, somewhat ill-defined, irregularly round shaped rings are seen. They are either exclusively or mainly composed of accumulations of fine, bluish reflecting particles. The center of these rings is sometimes empty, usually, however, filled by a small clump of the same bluish reflecting substance or of a mixture of the bluish and a coarse white reflecting matter. Small white reflecting particles occur also in some of the bluish rings. Reddish or brownish red reflecting granules are usually absent in the ring formations. They appear in only a few of the sections, where they participate in appreciable amounts in the composition of the rings and their central content in some areas. Surrounding the individual rings an indistinctly outlined area of less densely packed bluish granules fades away into the above described matrix.

The rings are much more distinct in those areas which show in the stained sections evidence of cell degeneration. They stand out as more or less solid, white reflecting formations, consisting either of a continuous or a discontinued, bead-like accumulation of coarse, white particles. The central portions of the rings are sometimes empty, but in general they are filled with the same white reflecting material of which the rings are composed. Solid

white, round bodies are often seen in such areas in place of ring formations and are apparently the result of a merger of the ring and its central content. White granules are also found in varying number in the zone surrounding the individual rings. There is not infrequently a second, outer ring present, consisting of white particles arranged in a bead-like formation which seems to demarcate the cell from the intercellular matrix. A larger amount of mineral matter, in which the white reflecting particles are prominent, is also found in the matrix.

The necrotic foci of the tumor tissue are characterized in the incinerated section by an incomplete or complete loss of the cellular structure. There are more or less dense, diffuse accumulations of white reflecting matter, which contains sometimes smaller and larger reddish brown particles.

As the interpretation and significance of the observations recorded depends to a certain extent upon the chemical nature of the various elements visible in the ash pictures, brief reference may be made to the information available at the present time on this subject. It is conceded by all investigators that the white reflecting particles represent calcium, probably in the form of calcium oxide. The brownish red to red granules seen in the ash are iron oxide. The nature of the bluish reflecting material is not definitely known. Policard suggested that it represents potassium and sodium. Scheid<sup>9</sup> has called attention to the presence of brownish, homogeneous, glassy or granular substances, which appear especially often in tissues containing large amounts of phosphatids, such as the white substance of the brain. These droplets, which may occasionally express a similarly colored liquid forming a network in the ash (artefact), are according to the investigations of Tschopp<sup>5</sup> and Scheid<sup>9</sup> phosphorus oxide. It may be mentioned in this connection that identical formations of droplet or network type have been observed in the incinerated sections of the two tumors examined and were found in general in close relation to degenerative and necrotic foci, where, however, also brownish red granules of iron character were frequently met.

#### COMMENT

The examination of the incinerated sections of the two transplantable rat cancers showed that the inorganic remains of the healthy tumor tissue are mainly bluish reflecting granules, which represent according to Policard's opinion sodium and potassium. Similar observations on the color of the ash of malignant and normal proliferating tissue were made by Olch<sup>10</sup> who reported that the ash of human breast and skin carcinomas and of the epithelial proliferations in chronic cystic mastitis, in hyperkeratoses and warts has a bluish color. Calcium appears either to be absent or to be present only in small amounts in the mineral skeleton of the healthy tumor cell. Iron does not seem to play any important rôle in the mineral constitution of the cytoplasm and the nucleus of the cells of the two tumors examined.

While the ring-like accumulations of mineral matter are located in the region where the nucleus is found in the stained sections, it appears to be doubtful, judging from the size and diameter of some of the rings, whether

these formations are located in the peripheral parts of the nucleus or are mineral matter absorbed to the nuclear surface or represent to a certain extent accumulations of minerals in the perinuclear space. These mineral rings can, however, not be looked upon as artefacts resulting from the action of fixating and dehydrating agents upon the cells, as suggested by some investigators.

These findings do not substantiate in some respects those recently made by Scott<sup>3</sup> and Scott and Horning,<sup>3</sup> who reported that malignant cells are characterized by an extraordinary concentration of iron and to some extent also of calcium in the peripheral margins of the nuclei resembling in this regard the mineral structure of embryonic cells. An explanation for this discrepancy cannot be offered at the present time. The only conclusion which can be justly drawn from the evidence at hand seems to be, that not all malignant cells resemble in their mineral structure that present in embryonic cells.

The observations made do not confirm another claim of Scott in regard to the presence of correlations existing between the mineral structure of the nucleus of malignant cells, the distribution of chromatin substance and the absorption picture obtained by ultraviolet ray photography. Scott has to a certain extent disproved his own contention in his experiments with opalinids, where he noted the absence of a relation between the scanty amount of nuclear ash and the good amount of chromatin present. The chromatin substance of nuclei of malignant cells in tumors in men, mice or rats is moreover not accumulated in the peripheral parts of the nuclei as contended by Scott. An accumulation of chromatin matter in this region is in general considered as evidence of cell degeneration. The location of the mineral substance as demonstrated by the incineration method does, therefore, not correspond to that of the chromatin matter of healthy tumor cells. The actual existence of the correlations cited above seems to be insufficiently supported by reliable experimental evidence.

In regard to the absolute amount of mineral matter in malignant cells, the investigations reported above do not support the conception that malignant cells have a higher mineral content than normal cells (Scott). This impression is gained by a comparison of ash pictures of areas of healthy tumor tissue with those of organs of similar cellularity, such as liver, spleen, pancreas, kidney, lymph node, salivary gland, etc. Much apparently depends upon the relative size of the cells and their functional state. Oleh<sup>10</sup> reported that the cells of the resting mammary gland contain large amounts of calcium. Policard and Pillet<sup>11</sup> stated that proliferating tissue has a low ash content and Policard and Doubrow<sup>12</sup> noted that the ash content decreases with the increase in the embryonicity of the tumor. Schultz-Brauns mentioned during a brief discussion of the ash content of various tissues that benign as well as malignant neoplasms show in general a light to moderate amount of ash. He added, however, that marked variations may occur among the ash content of individual tumors.

Considerable differences in the density of the mineral ash exist also between different parts of the same tumor. These have been referred to variations in the vital status of the cells. The ash and especially its calcium com-

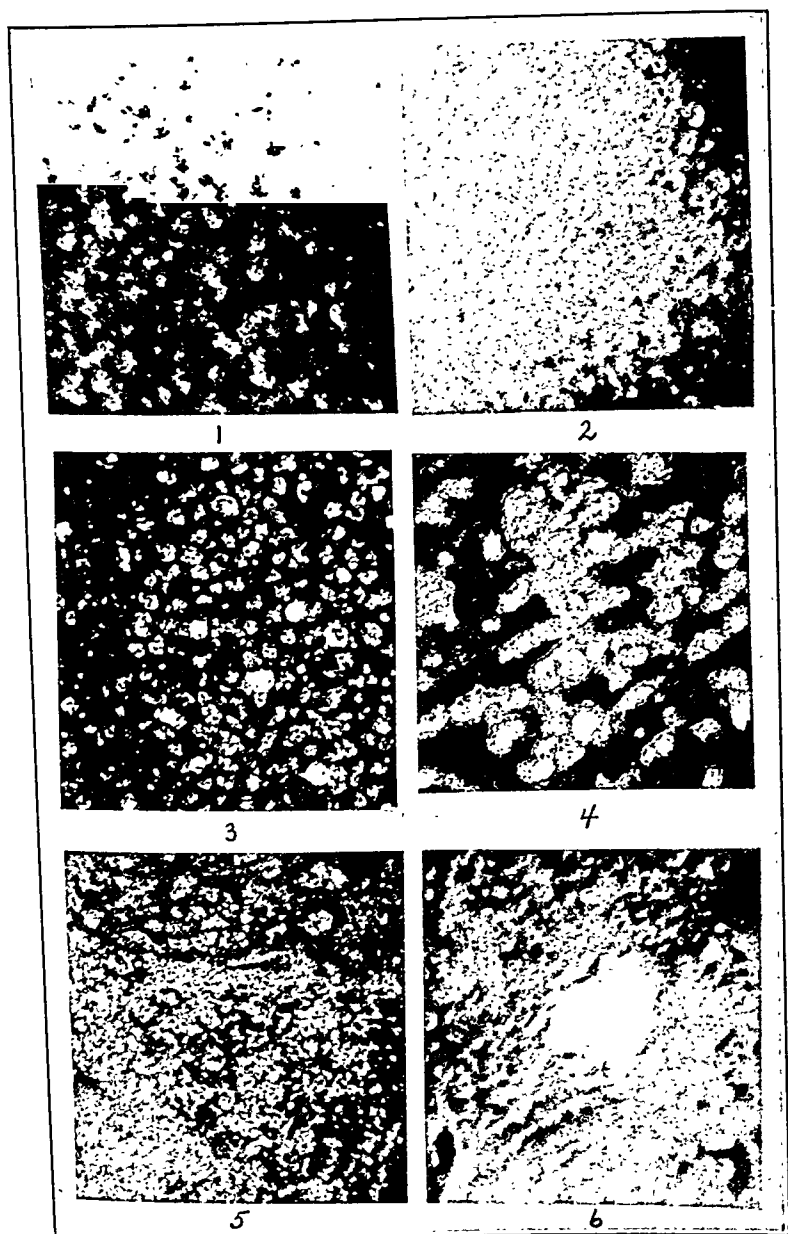


Fig. 1.—Incinerated healthy sarcomatous tissue showing rather faintly visible rings of bluish reflecting granules scantily mixed with white particles.

Fig. 2.—Ashed section of degenerating sarcomatous tissue with prominent white rings.

Fig. 3.—Degenerating sarcomatous tissue with white rings and central "calcifications."

Fig. 4.—Very edematous degenerating sarcomatous tissue with marked central "calcifications" and distinct white outer rings, demarcating the cellular outline.

Fig. 5.—Advanced degeneration of sarcomatous tissue with partial loss of cellular structure in the ashed section.

Fig. 6.—Necrotic focus in sarcomatous tissue characterized by dense accumulation of calcium particles.

ponent increases in direct proportion to the degenerative state of the cells and is apparently most abundant in areas of complete necrosis.

Shear<sup>11</sup> stated in his recent review on the rôle of different minerals (sodium, potassium, magnesium and calcium) in cancer, that it is the current view that the calcium content of tumors increases with increasing age and with increasing extent of necrosis. Policard and Doubrow<sup>12</sup> noted that degenerating and necrotic tumor tissue contains more ash than living tissue. Scott and Horning<sup>3</sup> remarked that their investigations furnished supplementary evidence for this observation. The experimental data obtained in this study confirm this conception and supply at the same time additional information in regard to the intracellular mineral changes and shifts which are associated with the process of degeneration.

An increase in the amount of calcium particles in the nuclear mineral ring represents apparently the first sign of a decreasing vitality and beginning degeneration of the cell. Following this nuclear calcinosis calcium particles are accumulated in increasing amounts in the central portions of the ring, until ultimately a union between the ash of the ring and of the central nuclear mineral deposits occurs. Accompanying this nuclear change there is also an increase in the number of calcium particles in the cytoplasmic region combined with a deposition of larger calcium granules in the peripheral parts of the cell (membrane?), where thereby a second outer ring is formed. With the ultimate complete disintegration of the cellular structure with progressing necrosis a definite mineral skeleton of the cells disappears and diffuse accumulations of mineral matter mainly representing calcium take their place. Older necroses are characterized by denser, almost solid masses of coarse calcium particles.

It was noted that necroses which showed in the incinerated sections marked deposition of calcium were not stained blue with the hematoxylin stain, but were pink, indicating thereby that the calcium present in these necroses is either too small in amount to be visible in the stained section or that it is present in a form which cannot combine with the hematoxylin. If the last conception should be correct the stainability of intracellular calcium would depend upon the physicochemical state in which this substance is held. A certain similarity would exist in this respect to the stainability of intracellular fat. As cellular degeneration is often accompanied by an accumulation of fat, correlations may exist between the increase of mineral ash, especially calcium, and that of fat in the degenerating cell (Schultz-Brauns). It is, however, doubtful, if an accumulation of calcium in the cell designates always a degenerative process or if it may also characterize a cell with low vital activities.

#### PART II. THE EFFECT OF PARATHORMONE AND VIOSTEROL MEDICATION UPON THE MINERAL CONTENT OF NORMAL AND MALIGNANT TISSUES IN RATS

Various authors have attempted to influence cancer growth by the introduction of substances which affect the calcium metabolism, such as calcium salts, Irradiated ergosterol and parathyroid hormone, into the cancerous organism

(Goldfeder,<sup>14</sup> Paik,<sup>15</sup> Barelli,<sup>16</sup> Sugiura and Benedict,<sup>17</sup> Goerner,<sup>18</sup> Goerner and Shafroff,<sup>20</sup> Sumi,<sup>19</sup> Bischoff and Maxwell,<sup>21</sup> Reding and Slosse<sup>22</sup>). Shear<sup>13</sup> came, in his recent review on this subject, to the conclusion that parathormone treatment is ineffective as an agent which may inhibit or stimulate malignant growth and that vitamin D is without any reliable antigrowth action. This conception is confirmed by observations made by me some time ago on a larger series of cancer patients who received calcium salts, viosterol and parathormone over a prolonged period and in relatively large doses.

In view of the controversial observations made on the blastic action of vitamin D and parathormone, a histochemical study of ashed sections prepared from cancer tissue of animals, treated with these two agents, seemed to be of interest, as it could be assumed that any action of these substances on the tumor would very likely be associated with definite changes in the mineral constitution of the cells.

Six rats, three inoculated with the transplantable sarcoma, Philadelphia No. 1, and three inoculated with the transplantable carcinoma, Walker No. 256, were used in the parathormone experiment. After the tumors had reached the approximate size of a walnut and after a small piece of the tumors had been removed for comparative study, the rats received subcutaneous injections of parathormone. One animal was killed twenty-four hours after the injection of 50 units of parathormone; three rats were killed sixteen hours after the injection of 100 units of parathormone. Rat 5 was sacrificed sixteen hours after injection of 150 units and Rat 6 received a total of 250 units in the course of three days and died on the fifth day after the beginning of the medication.

As the rat is rather resistant to parathormone (Collip,<sup>23</sup> Tweedy and Chandler,<sup>24</sup> McJunkin, Tweedy and Breuhaus<sup>25</sup>), relatively large doses of parathormone are required for the production of toxic degenerative organic lesions, such as those rather readily obtained in dogs (Hueper).

In the viosterol experiment six rats with sarcoma, Philadelphia No. 1, were fed with 0.75 c.c. of viosterol 10,000 X daily by mouth with a dropper. After five days of this medication the rats became listless and showed a coarse fur. The treatment was discontinued and one animal was killed four days later. After an interval of a week the medication was resumed for a period of four days. One animal died two days after the resumption of the feeding and one was killed at that time. The remaining three animals were killed ten days later, while they were still showing symptoms of viosterol intoxication.

Any inhibitory effect of the viosterol medication upon the growth rate of the tumors was not observed. This may be noteworthy, in spite of the small number of animals used, on account of the rapid growth of these neoplasms.

The autoptic examination of the rats did not show any macroscopic pathology in the tumors and organs which might have been referred to the medication given, with exception of Rats 5 and 6, where irregular whitish, soft areas and streaks were found on the cut surface of the kidney and heart, indicating the presence of necroses.

The histologic findings in the organs of the rats in both groups were essentially identical. The following organs were examined: tumor, salivary gland, heart, lung, liver, pancreas, spleen, kidney, lymph node, suprarenal gland, and skeleton muscle.

The stained and incinerated sections prepared from the tumors of the

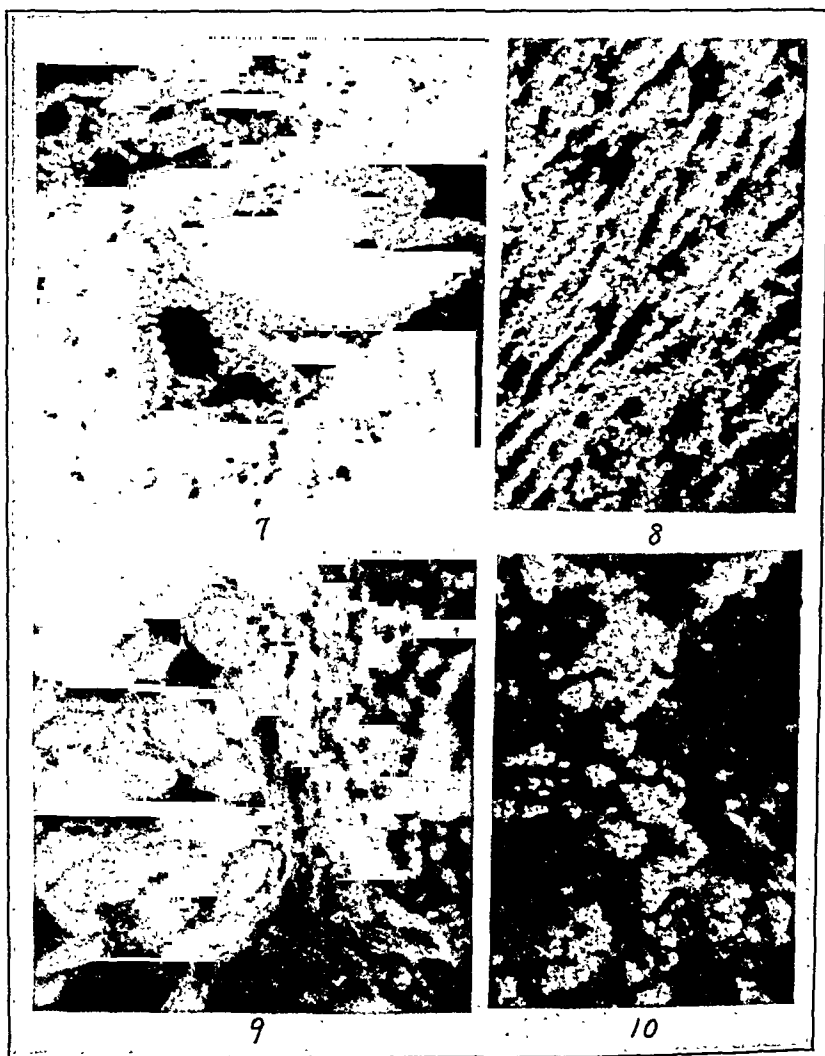


Fig. 7.—Vascular and myocardial calcification in the heart after parathormone poisoning.

Fig. 8.—Accumulation of calcium particles in the myocardium without evidence of degenerative changes in the stained section.

Fig. 9.—Accumulation of calcium in the muscularis of the esophagus of Rat 6, which died from parathormone poisoning.

Fig. 10.—Calcifications in the kidney of Rat 6.

treated animals did not reveal any appreciable and fundamental difference from those made before the medication was started. There were no changes in the stainability of the cytoplasm and of the nucleus of the tumor cells. The ashed sections were essentially identical with those previously described.



There was neither an increase nor a decrease of mineral matter, especially of calcium, in the tumor cells and the intercellular spaces.

In contrast to these negative results in the tumors definite histologic and histochemical pathology was found in some of the organs of the treated rats, especially in Rats 5 and 6, indicating that the medication given had been intense enough to cause pathologic lesions in some parts of the organism.

*Heart*.—There is a moderate to marked interstitial and especially perivascular leucocytic infiltration of the myocardium. A bluish stained hyaline material is found here and there in the perivascular spaces in between the leucocytic exudate. The walls of the myocardial vessels of Rats 5 and 6 are markedly thickened and deeply blue stained. Reddish and blue stained necroses surround and extend from these vessels into the myocardium. The incinerated sections show a moderate, spotty increase of white reflecting particles. The leucocytic infiltrations appear as small accumulations of strongly reflecting white dots. The vascular walls in the heart of Rats 5 and 6 appear as thick, solid white rings, from which less massive accumulations of white matter extends into the surrounding ash which is also rich in white reflecting granules.

*Esophagus*.—The muscularis of the esophagus of Rat 6 is dark grayish blue in color and contains dense accumulations of calcium particles in the ashed section.

*Liver*.—In some of the livers examined the cytoplasm of the Kupffer cells and of scattered individual liver cells is blue stained. The distribution of accumulations of white granules corresponds approximately with that of these bluish stained cells. There is a general increase of white ash. It is noted that the liver cell areas surrounding the central veins do not participate in this increase, but are composed of bluish and reddish reflecting ash.

*Kidney*.—The tubular epithelium of the cortex shows a distinctly bluish stained substance located in the pink stained cytoplasm. This bluish material surrounds sometimes the nucleus and exudes and fills the lumina of the tubules. Small, isolated foci of necroses are occasionally seen. In the kidneys of Rats 5 and 6 extensive necroses of the tubular epithelium are present. There are also localized calcifications of the necroses and calcium casts in the tubules in great number. The walls of many of the smaller vessels are thickened and intensely blue stained, indicating a high degree of calcification.

The ashed sections show a marked increase of white reflecting material, which often forms dense clumps or rings corresponding in their distribution with that of the tubular necroses and vascular calcifications, respectively, seen in the stained sections.

The microscopic examination of the stained and ashed sections of the other organs mentioned above did not reveal any differences from the conditions seen in the sections of a series of three normal rats of the same strain and approximate age as the experimental animals.

#### COMMENT

The observations made seem to indicate that the cells of the two rat tumors used do not belong to that group of tissues in which disturbances of the mineral metabolism caused by overdosage of parathormone and viosterol, respectively, result in local degenerations and necroses. The absence of any appreciable changes in the amount, character, and distribution of the mineral matter of the cancer cells following the medication of parathormone and viosterol suggests that the malignant tissue is apparently unable to withdraw calcium from the hypercalcemic blood, because the calcium is present in the blood in a form which cannot be taken up by the malignant cells. This explanation is supported by the observations of Kluge<sup>27</sup> who found, that there is a marked difference in the absorption of calcium compounds by tumor tissue

depending on their organic or inorganic character. While the subcutaneous injection of inorganic calcium compounds resulted in a marked temporary increase of the calcium content of the tumor tissue, its calcium content was barely increased when organic compounds were used.

The presence of a bluish stained substance in the perivascular spaces of the myocardial vessels and in the cytoplasm of the renal epithelium and liver cells together with the corresponding increase of calcium particles in these cells according to the observations made in the ashed sections suggests, that intracellular disturbances of the mineral metabolism precede and cause the degeneration and necroses of the myocardium and kidney occurring in parathormone and viosterol poisoning. This conception receives additional support by previous investigations of Hueper,<sup>7</sup> McJunkin, Tweedy and Breunhaus<sup>25</sup> and Ham.<sup>26</sup> It is obvious that the lethal cellular calcinosis may not always be followed and intensified by an additional necrotic calcification. The presence of a hypercalcemia will, however, favor this process. The contrast existing between the marked increase of calcium in degenerative and necrotic foci, according to the ashed sections, and the absence of a positive staining reaction for calcium in these areas, according to the stained sections, seems to suggest the possibility that the demonstration of calcium in the tissue with the hematoxylin stain depends not only on the amount but also on the character of the calcium compound present.

#### CONCLUSIONS

1. Microincineration of two transplantable rat cancers showed that the healthy tumor cells consisted almost exclusively of bluish reflecting granules, representing presumably sodium and potassium.

2. Iron and calcium did not seem to play any important rôle in the mineral constitution of these cells.

3. Cellular degeneration in these two tumors was characterized by an increase of the calcium content of the cells, manifested mainly in the formation of white nuclear rings.

4. The character and composition of the mineral skeleton of the tumor cells could not be changed by the medication of parathormone and viosterol.

5. Degenerations and necroses in the kidney and heart of rats receiving excessive amounts of parathormone and viosterol were apparently caused by the toxic action of an intracellular calcium accumulation.

I am indebted for technical assistance to Miss M. Russell, Cancer Research Laboratories, University of Pennsylvania.

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# THE SIGNIFICANCE OF STREPTOCOCCI THAT RESEMBLE DIPHTHEROIDS RECOVERED FROM BLOOD CULTURES IN SUBACUTE BACTERIAL ENDOCARDITIS\*

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AN ASTONISHING change in size and shape of streptococci may be noticed under different conditions of cultivation.<sup>1</sup> At times these changes result in forms that have a distinct resemblance to the diphtheroid group of bacilli. In old cultures the changes that take place are probably best interpreted as "involution forms." The term "diphtheroid" is applied to an ill-defined group of bacilli, largely on account of their morphologic resemblance to the diphtheria bacillus. In this article the terms "diphtheroid" or "diphtheroid appearing" are used because of the morphologic resemblance of the organisms studied to the diphtheria bacillus and do not specify any particular bacterium.

Jensen and Morton<sup>2</sup> have, from a case of acute cystitis, isolated a streptococcus that under certain conditions resembled a diphtheroid bacillus. They speak of the organism as having a diphtheroid and a streptococcus phase.

Tow and Wechsler<sup>3</sup> have reported a case of acute endocarditis in a girl eleven years old in which diphtheroids were recovered from the blood during life and at autopsy. One year prior to the onset of the acute illness the girl had pain in the right knee and fever which lasted one week. Eight blood cultures during the course of the disease were negative, and the ninth was positive for a diphtheroid organism. Sections of the vegetations taken at autopsy showed cocci in pairs and groups. The vegetations were not cultured. In their conclusion Tow and Wechsler state that the existence of diphtheroid endocarditis is problematical and that it is likely that the streptococcus was the etiologic agent and the diphtheroid a secondary invader.

According to Thomson and Thomson<sup>4</sup> "true diphtheroids" do not show streptococcal forms in broth cultures. If this statement is true then it is of unusual interest to find diphtheria-like forms and typical streptococci in smears from uncontaminated young broth cultures from the blood of a patient suspected of having subacute bacterial endocarditis; further to find similar forms in a (Gram-Weigert) stained section from the vegetations on the heart valve when the patient came to autopsy.

## REPORT OF CASES

CASE 1.—*History:* L. H., twenty-three years of age, admitted to The Meriden Hospital, Jan. 31, 1933, complaining of weakness and fever. Had rheumatic fever in 1921. About two weeks prior to admission, patient had grip, on fifth day pain developed in right shoulder,

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and persisted for three or four days, then disappeared. Had one attack of nosebleeding before admission. Had severe headache. Had palpitation and dyspnea occasionally. *Examination:* Throat slightly injected. Tongue coated. X-rays showed one abscessed tooth. Heart enlarged to the left; the point of maximum impulse was in the sixth interspace anterior axillary line. A presystolic thrill was felt. Diastolic murmur along the left border of the sternum most marked at the apex and referred to the left axilla. Lungs were clear. Spleen was felt at the costal margin. On February 14, embolic spots were found on finger tips. February 18, abscessed tooth was extracted. From this was cultured nonhemolytic, green pigment-producing streptococcus.

In view of favorable reports by Brody and Crocker,<sup>5</sup> Gordon,<sup>6</sup> Stephenson<sup>7</sup> and Howell, Portis and Beverly,<sup>8</sup> an immunotransfusion was decided upon. A vaccine was prepared from the organism found in the patient's blood stream. A suitable donor was given six injections of the vaccine subcutaneously, and one injection intravenously. Following the last injection he had a severe reaction with chills and fever.



Fig. 1.

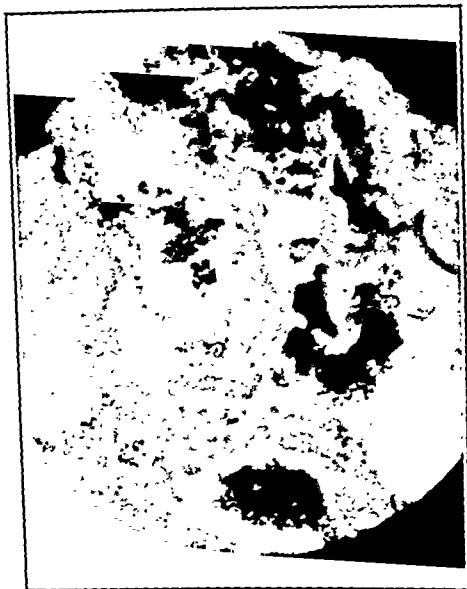


Fig. 2.

Fig. 1.—Shows enormous masses of bacteria in the vegetation on the aortic valve. Gram-Weigert stain.  $\times 100$ .

Fig. 2.—Section from the vegetation on the aortic valve stained by Gram-Weigert method. Shows an abundance of diphtheroid organisms and one chain of streptococcus.  $\times 1000$ .

Six days after the last injection was given to the donor, the patient was given 300 c.c. of whole blood. Following transfusion he developed cyanosis, dyspnea, and cough. This critical condition persisted for several hours. He was slightly dyspneic for two or three days. From then on his condition became gradually worse, and he died on March 20. The temperature was irregularly high, ranging from  $90^{\circ}$  to  $104^{\circ}$  from admission until four days before death, when it fluctuated between  $96^{\circ}$  and  $98^{\circ}$ .

*Laboratory Observations:* Wassermann negative. Blood nonprotein nitrogen 24 mg. Blood sugar 120 mg. Several blood counts were done. The erythrocyte count varied between 4.4 millions on February 1 to 3.6 millions on March 6. The leucocytes ranged from 12,200 on February 1 to 16,600 on March 6. The differential remained fairly constant. Hemoglobin 75 per cent; polymorphonuclear neutrophils 74 per cent; small lymphocytes 17 per cent; large lymphocytes 12 per cent. The blood culture was positive.

## AUTOPSY

*Gross Observations:* Well-developed and nourished adult male. Petechiae observed on extremities and abdomen. Finger nails, lobes of ears and lips deep bluish in color. Each pleural cavity contained about 500 c.c. of clear, straw-colored fluid and a few flakes of fibrin.

*Heart:* Weighed 500 gm. The myocardium was soft and pale. The pulmonary and tricuspid valves appeared normal. The free margin of the mitral valve was slightly thickened and whitish in color. There were three pale grey, friable vegetations measuring 0.3 cm. in height by 0.3 cm. in diameter at the base of the right cusp of the mitral valve. The vegetations were located on the ventricular surface of the valve. The columnae carnae were flattened and some chordae tendineae showed slight fibrosis. The cusps of the aortic valve were obscured by a large, pale grey, friable vegetation. All the cusps were involved. The great mass of the vegetation was on the ventricular surface of the valve. The cusps were not ulcerated, although they were considerably distorted in shape. The remaining organs showed the usual appearance as found in subacute bacterial endocarditis.

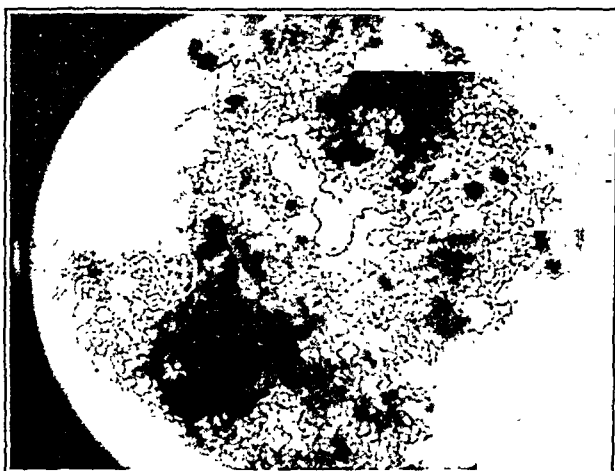


Fig. 3.—Smear from broth culture which was planted from a blood agar plate. Shows a marked predominance of long chain streptococcus.  $\times 1000$ .

## MICROSCOPIC EXAMINATION

Section through the aortic valve showed a massive vegetation composed of polymorphonuclear leucocytes, fibrin, platelets, red blood cells, and dark blue staining masses of bacteria. A Gram-Weigert stained section from aortic valve showed great masses of cocci in chains and diphtheroid appearing organisms. The microscopic examination of the remaining organs showed nothing of special importance.

*Summary:* The important findings in this autopsy were vegetative endocarditis involving both aortic and mitral valves, multiple petechiae over the body, infected infarcts of the spleen, chronic passive congestion of the liver and acute congestion of the lungs, spleen, and kidneys.

*CASE 2.—History:* F. G., unmarried female, nineteen years of age, was a dramatic art teacher. The significant features in the past history were scarlet fever at seven and chorea at ten years of age. Her last illness began in September, 1932, when she complained of pain in hips and ankles and went to bed for three weeks at home. On getting up she had pain in her back and had an enlarged liver and spleen.

She was a patient in the New Haven Hospital, New Haven, Conn., in November, 1932. A blood culture on Nov. 3, 1932, was positive for diphtheroids. The patient left the New Haven Hospital in December, 1932, against advice. The diagnosis on the record was ?

subacute bacterial endocarditis.\* She was admitted to the Meriden Hospital, Dec. 29, 1932, and discharged Jan. 14, 1933, on request of the family. During this period four blood cultures were taken with negative results. Following discharge from the hospital, patient was admitted several times for blood transfusions and usually remained in the hospital a day each time. On April 25, 1933, a blood culture was positive for diphtheroids. The bacteriology of these diphtheroid-like organisms was similar to the bacteriology of those described in the case reported above. Patient died in May. An autopsy was refused.

#### BACTERIOLOGIC STUDIES

Three cultures were studied in this series. The first (laboratory number 3,674) was isolated from a blood culture during life in patient in Case 1, the second (laboratory number 7,038) was isolated from heart's blood at autopsy; the third (laboratory number 14,722) was isolated from blood of patient in Case 2 during life. After isolation several platings on blood agar were made and in each instance single colonies chosen to rule out possibility of contamination. Culture (laboratory number 14,722) was the same in all respects as Culture 3,674, so for the sake of brevity a description of the experimental studies on this culture are omitted.

*Morphology.*—The morphology of all three cultures was studied when grown on Loeffler's medium, blood agar plates (both aerobic and anaerobic) and in Douglas broth.

On Loeffler's medium culture 3,674 was a mixture of gram-positive streptococci in association with diphtheroid, bacillary-like forms (narrow, club-ended, and granular). There was a tendency for the latter forms to decolorize with the Gram stain. Culture 7,038 was similar microscopically except that the streptococci and paired forms predominated in young cultures. Cultures five days old, however, showed many diphtheroid forms.

The growth on blood agar plates of Culture 3,674 (aerobic and anaerobic) was fine and veil-like in character. In forty-eight hours a small zone of hemolysis appeared. By repeated incubation at 37° C. followed by refrigeration, secondary hemolytic rings were developed. The hemolysis was typical of the alpha variety.<sup>9</sup> Both diphtheroid and streptococcus forms were demonstrated microscopically.

The beta type of hemolysis was produced with Culture 7,038 when grown on blood agar plates either aerobically or anaerobically. Individual colonies were larger than with Culture 3,674 and showed wide, clear zones of hemolysis. After a series of eight daily transplants a few alpha type colonies developed which retained their alpha characteristics on subculture. Both diphtheroid and streptococcus forms were demonstrated microscopically.

Transplants to Douglas broth caused practically complete change to the streptococcus phase of both Cultures 3,674 and 7,038 regardless of whether inoculum was obtained from Loeffler's slants or blood agar plates. Continued daily transplants over a period of forty days were made and without exception a pure culture of long-chained streptococci (16-18 cocci to a chain) was obtained. The streptococci remained constant in their morphology for three

\*We are indebted to the Department of Internal Medicine of Yale University for permission to use data from records of the New Haven Hospital.  
The microphotographs were taken by Mr. Jean Kiefer of The Norwich Tuberculosis Sanatorium.

to four days. Usually between the fifth and seventh day diphtheroid forms could be demonstrated microscopically as the chains of streptococci appeared to have changed to the diphtheroid phase. Transplants at this point to fresh Douglas broth, however, again resulted in a pure culture of streptococci. There was a general trend toward the diphtheroid phase in cultures as they grew older. In no instance was the reverse of this phenomenon noted in any medium upon aging.

*Biochemical Reactions of 3,674 and 7,038.*—Both cultures were inoculated into 1 per cent sugar broths from Douglas broth, Loeffler's slants, and blood agar slants. Sugar broths were sterilized by filtration. Both cultures, 3,674 (alpha hemolytic) and 7,038 (beta hemolytic), gave similar reactions: Dextrose, lactose, sucrose, salicin and maltose were fermented with the production of acid. Mannite, rhamnose, arabinose and raffinose were not affected although definite growth was obtained. In litmus milk, acid and coagulation was produced. On the basis of the morphologic and biochemical reactions Culture 3,674 would be classified as a strain of *Streptococcus viridans*, variety probably *mitis*. Culture 7,038 would be classified as a strain of *Streptococcus hemolyticus*.

#### SEROLOGIC STUDIES

In order to augment our findings both cultures were sent on blood agar slants to the Rockefeller Institute\* for further study. The following report was obtained:

"Culture 7,038 seems to be a typical hemolytic streptococcus. A precipitin test with an extract of the organism shows that it contains the carbohydrate 'C,' characteristic of strains of human origin (Group A).

"Culture 3,674 seems to be a typical *Streptococcus viridans* and not merely a non-hemolytic form of 7,038, since extracts of 3,674 do not show any traces of the 'C' substance characteristic of hemolytic streptococci belonging to Group A."

After several weekly transplants on Loeffler's slants and blood agar plates, Culture 3,674 developed colonies which showed typical beta hemolysis. When single colonies were chosen and plated on blood agar, however, both alpha and beta type colonies developed. Morphologically and biochemically both types of colonies (alpha and beta) agreed with the colonies obtained from Culture 7,038 (alpha and beta). These four cultures were then sent to the Rockefeller Institute and tested for the group carbohydrate "C." The two beta hemolytic strains both gave precipitin reactions with anti "C" serum for Group A and no reaction with anti "C" serum for any of the other groups. The two alpha hemolytic strains did not react with any of these sera. From these studies it is obvious that the colonies showing alpha hemolysis are antigenically distinct from those showing the beta type.

#### COMMENT

The interesting features of the case clinically were: the reaction following transfusion with a specifically immunized donor; and the blood culture studies.

\*The authors are indebted to Dr. R. C. Lancefield for these studies.



Two possibilities present themselves as probable causes of the severe reaction following immunotransfusion. First, the occurrence of a pulmonary embolus following the transfusion, always a possibility, must be considered. Second, the interaction between the antibody of the donor and antigen of the recipient. Of these two possibilities, the latter appears to us to be the more likely. The patient's tissues were proliferated with antigen. Both the streptococcus and diphtheroid phases were demonstrated in tissue section at autopsy. The donor received both phases in the vaccine used for production of the therapeutic serum. With a preponderance of antigen in his tissues, the recipient was in a highly sensitive condition and the violent reaction following transfusion with whole blood containing antibody was probably due to an antigen antibody complex in the tissues and blood stream of the patient. The clinical picture of cyanosis, dyspnea and cough directly following the immunotransfusion would appear to augment this theory.

Jensen and Morton's<sup>2</sup> experiments with the diphtheroid phase of a streptococcus isolated from a case of acute cystitis led them to believe that the diphtheroid phase was nonpathogenic. We are carrying on experiments at the present time that appear to show that in rabbits the diphtheroid phase is pathogenic in the sense that definite valve localization can be produced. At this time, however, we wish to suggest the possibility that in certain instances, in subacute bacterial endocarditis, it is the attenuated diphtheroid phase of the streptococcus that first becomes implanted on the heart valve. Once a small clot is formed on the valve the medium is perhaps suitable for the change of the organism into the streptococcus phase following which the disease progresses in the usual manner.

In a study of 1,079 blood cultures Thompson<sup>10, 11</sup> has shown the presence of diphtheroids in 3 per cent of those examined. His object was to test the hypothesis advanced by Koch and Mellon<sup>12</sup> that diphtheroids are found in the blood stream in persons convalescent from streptococcal infections. Thompson believes that diphtheroids in blood cultures appear to have the same significance as the saprophytic cocci.

Thompson<sup>10, 11</sup> states that "Any organism which appears as rods, and which in subcultures in broth develop chains of streptococci may better be classified as streptococci, especially if it is not possible subsequently to bring about the reverse change from the streptococcal to the rodlike form." The organism numbered 3,674 in this investigation originally showed a majority of diphtheroid forms and subsequently developed into a streptococcus, but we have been able to change this organism back to the diphtheroid phase at will by the use of proper media.

If we accept the hypothesis of Thomson and Thomson<sup>4</sup> the organism would have to be classified as a streptococcus with a diphtheroid phase. However, whether we regard this phenomenon as one of pleomorphism or not is not our basic interest. We are primarily interested in focusing attention on the significance of the finding of diphtheroid appearing organisms in blood cultures, particularly in cases of subacute bacterial endocarditis.

Grinnell<sup>13</sup> reports that he was able to obtain from single cell strains of *Streptococcus hemolyticus*, strains of *Streptococcus viridans* with constant char-

acteristics. He notes "the alpha variants remain true to type, the loss of hemolytic power is accompanied by a slight loss of virulence, the alpha derivatives usually but not always give the same fermentative reactions." In this investigation we were able to confirm these results. The alpha variants from the beta hemolytic types were antigenically distinct although giving identical fermentative reactions.

Our experiments on animals to date have shown definitely that the organism has very low virulence. Large quantities, regardless of the phase, when injected into rabbits produce no toxic effect. However, by repeated injections of any of the three phases over a period of from twenty-three to thirty-five days definite localization has been demonstrated. This section of the investigation has not yet been completed.

#### SUMMARY

1. Two cases of subacute bacterial endocarditis are described in which diphtheroid appearing organisms were recovered repeatedly from blood cultures during life.

In Case one, Gram-Weigert stained sections from the vegetation on the aortic valve at autopsy showed both diphtheroid appearing forms and chains of streptococci in each section. Bacteriologic investigation in both cases showed that the streptococcus under study existed in two phases, a typical streptococcus phase and a diphtheroid phase.

2. As a result of the bacteriologic studies of the two cases reported in this paper evidence is submitted which shows that the occurrence of diphtheroid appearing organisms in blood cultures from suspected cases of subacute bacterial endocarditis must be regarded as important. The organism, isolated from blood cultures in the diphtheroid phase, changed under suitable conditions into a green producing streptococcus which is unquestionably of etiologic significance.

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# THE USE OF GALACTOSE IN THE DIFFERENTIAL DIAGNOSIS OF JAUNDICE\*

## A PRELIMINARY REPORT

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IN A SURVEY of the literature concerned with the physiology of the liver and the tests devised to estimate its functional capacity,<sup>1, 2, 3, 4, 5, 6</sup> it is noteworthy that until recently very little of clinical value has been accomplished either in the diagnosis or in the prognosis of liver disease.

There are several reasons for this. First, the liver is a huge organ, the site of multiple activities, and has been aptly called "the great storage battery of the human body." It is obvious, therefore, that no one test is likely to yield an accurate index of its total functioning capacity. Furthermore, experimental work has shown that there is a tremendous hepatic reserve, a reserve greater probably than in any other organ of the body. To quote from the report of Bollman and Mann on lesions of the liver produced experimentally in dogs:<sup>5</sup> "It would appear that any test, capable of measuring the metabolic changes which depend entirely on the liver, would give an accurate index of the efficiency of this organ. There are several reasons why such tests show but little, and studies of animals from which the liver has been partially removed reveal some of the reasons for the failure of those tests. In the dog and in many other animals, the liver consists of several distinct lobes, one or more of which may be successfully removed. Immediately following such operations, the remaining portions of the liver begin to hypertrophy, and by serial operations, removing a lobe of liver every few weeks, it is possible to remove more hepatic tissue than was originally present and still have a normal amount of hepatic tissue remaining in the dog. During this process no signs of hepatic insufficiency are observed except a transient upset of a few days' duration, which is probably due to injury of its remaining hepatic tissue rather than to actual deficiency because of removed tissue. . . . It is obvious that less than 20 per cent of liver can accomplish the work of the entire organ."

Second, the main effort and emphasis in the study of this problem from the laboratory standpoint have been expended on attempting to estimate the excretory capacity or permeability of the liver by the use of various dyes—in a way analogous to the dye tests of renal function. As a result of this, the bromsulphalein and rose bengal tests have come into general clinical use. Their value is indeed very limited, for while the rate of their elimination from the blood stream and excretion by the liver may give some information as to the permeability of the liver and the patency of its biliary system, it helps not at

\*From the Cleveland Clinic.

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all in estimating the ability of the liver to perform its more vital functions. Foremost among these may be mentioned the conversion of sugar to glycogen, its storage and release again into the blood stream as glucose; the synthesis of urea; the formation of bile pigment and the detoxification of poisons.<sup>6</sup> The dye excretion tests do not indicate the capacity of the liver to perform these functions; and furthermore, when either intrahepatic or obstructive jaundice is present, the estimation of dye in the plasma is bound to be inaccurate, yielding a value which is almost always too high.

Tests of pigmentary function of the liver, of which the van den Bergh test is outstanding, have been of no help in determining the nature or extent of liver damage and have failed particularly in the differentiation of obstructive and intrahepatic jaundice. In recent years, the rate of removal of bilirubin injected intravenously has been used as a test of liver function as reported by Bergmann in 1927 and by Harrop and Barron in 1931.<sup>7</sup>

Estimation of the cholesterol content of the blood plasmas in cases of hepatic and biliary disease has been reported recently in an excellent article by Epstein.<sup>8</sup> This promises to be a valuable means of estimating liver damage.

Since the discovery of the glycogenic function of the liver by Bernard about the middle of the last century, many reports have been published in the literature on the utilization of various sugars for testing hepatic function. These investigations have been productive of widely divergent results and interpretations, due to the fact that the tests were not standardized, that they were used in all varieties of liver disease, and that no allowance was made for the properties of great reserve and rapid regeneration which we now know to exist. In short, extensive acute or subacute damage to the liver must be present before changes are reflected by a derangement of carbohydrate metabolism.

In this report, I shall confine the discussion to that puzzling group of cases of relatively painless acute jaundice in which neither the history, physical signs, nor laboratory reports make the diagnosis clear. These cases may be due to common duct obstruction of one sort or another or may be caused by acute or subacute diffuse liver damage, such as occurs in catarrhal icterus or toxic hepatitis.

Recently a modification of the glucose tolerance test has been used with some success to estimate the liver function by workers at the University of California.<sup>9</sup>

Keeping in mind the functional complexity of the liver, its great reserve and power to regenerate rapidly, and the present inadequacy of the tests in general use to determine its "vital capacity," the problem of jaundice and its differential diagnosis by means of galactose tolerance may be considered.

This differentiation has often confused the keenest clinicians, yet it is of the utmost practical importance to the patient, since the therapeutic measures are diametrically opposed. Extrahepatic obstructive jaundice is a surgical problem. In jaundice due primarily to inflammation or degeneration of the liver parenchyma, surgery is positively contraindicated. The galactose tolerance test of liver function furnishes a simple and positive means of making this differentiation.

## USE OF GALACTOSE

About thirty years ago the use of levulose as a test for liver function was attempted by Strauss, and five years later Richard Bauer of Vienna began to use galactose for the same purpose.<sup>10</sup> Since that time levulose, dextrose, and fructose all have been proposed and tried as tests of the carbohydrate metabolism of the liver with little success. Galactose, which appears to be the most valuable of the monosaccharides for this purpose, has received but little attention in this country.

In 1931 several papers were published by Shay, Schloss, Bell, and Rodis of Philadelphia outlining the theoretical considerations for the use of galactose as a test of liver function<sup>11</sup> and also their results in cases of jaundice.<sup>12</sup> The work here reported was done in order to confirm or deny their findings.

From a theoretical standpoint, in considering the use of any carbohydrate as a test for liver function, galactose seems best suited for this purpose. First, the sugar must be pure and stable. When given orally it must be absorbed from the gastrointestinal tract easily and with little decomposition by bacteria. It must be capable of transformation by the liver into glycogen, preferably with difficulty; the normal tolerance for the sugar must be known; and finally, there must be a stable renal threshold.

Galactose may be obtained in a pure form, though it is rather expensive. It is rapidly absorbed from the gastrointestinal tract, and, according to present knowledge, it is utilized *only* by the liver and that with difficulty. It is well known that the other sugars have no such specificity. Furthermore, experimental work has shown that after reaching the general circulation it is all excreted by the kidneys, regardless of the condition of the other glands. There is no renal threshold for galactose.<sup>11</sup>

Since the liver has such adequate reserve and such power for rapid regeneration, it is clear that in local or slowly progressive liver disease there is no change in the carbohydrate metabolism. But it is equally obvious that marked derangement of carbohydrate metabolism might be expected when there is extensive acute or subacute damage to the liver cells or when chronic damage has gone on to such a stage that regeneration fails. The variable results which have attended many types of tests for the function of sugar metabolism may be explained partly on this basis. In other words, the test has definite limitations, and it is these very limitations which make it valuable in the differential diagnosis of jaundice. The galactose tolerance test of liver function serves to identify and differentiate those cases of diffuse liver damage which occur in toxic or infectious jaundice from those cases of jaundice due to obstruction of the bile ducts.

## TECHNIC OF THE GALACTOSE TOLERANCE TEST

The work of Shay and Schloss has shown that the normal liver, regardless of age, sex or condition of the other glands, can metabolize about 40 grams of galactose. That portion not stored by the liver appears in the urine as a reducing substance, identified chemically and polariscopically as galactose. In their series of normal persons, this output varied from 0 to 3 grams.

The test is carried out as follows:

1. A fasting specimen of urine is obtained.
2. The patient is given 40 grams of pure galactose, dissolved in one pint of water.
3. Breakfast is omitted.
4. All urine voided in the next five hours is collected, preferably in hourly specimens.
5. The specimens are tested qualitatively for galactose and all those positive are lumped in one volume and tested quantitatively by the Benedict method. (In diabetic patients it is possible to eliminate glucose by the process of fermentation with yeast, as galactose is relatively stable.)

In Table I are listed the series of normal values prepared by Shay and Schloss and my own series of normal values, obtained by the same technic. In respective columns are given the ages of these persons in years, their weight

TABLE I  
COMPARATIVE STUDY OF EXCRETION OF GALACTOSE IN MALES AND FEMALES  
*Shay et al.*

| FEMALES      |                 |                          | MALES        |                 |                          |
|--------------|-----------------|--------------------------|--------------|-----------------|--------------------------|
| AGE IN YEARS | WEIGHT (POUNDS) | GALACTOSE IN URINE (GM.) | AGE IN YEARS | WEIGHT (POUNDS) | GALACTOSE IN URINE (GM.) |
| 5            | 52              | 0.50                     | 6            | 36              | 0.50                     |
| 26           | 100             | 0.00                     | 52           | 110             | 1.20                     |
| 55           | 102             | 0.25                     | 64           | 110             | 1.50                     |
| 64           | 107             | 2.20                     | 53           | 112             | 1.08                     |
| 63           | 108             | 0.69                     | 59           | 115             | 0.30                     |
| 55           | 110             | 0.13                     | 42           | 120             | 2.20                     |
| 42           | 110             | 1.00                     | 30           | 126             | 1.00                     |
| 63           | 115             | 0.30                     | 64           | 129             | 0.00                     |
| 49           | 115             | 0.70                     | 50           | 129             | 1.20                     |
| 64           | 134             | 0.98                     | 50           | 133             | 1.10                     |
| 35           | 150             | 0.00                     | 40           | 142             | 1.20                     |
| 18           | 153             | 0.65                     | 69           | 160             | 0.00                     |
| 38           | 162             | 1.98                     | 59           | 162             | 1.10                     |
| 50           | 175             | 0.00                     | 51           | 162             | 1.87                     |
| 32           | 185             | 0.19                     | 54           | 166             | 0.00                     |
| Average      |                 | 0.64                     | Average      |                 | 0.88                     |

*Author*

|               |              |      |
|---------------|--------------|------|
| 20<br>↓<br>70 | 0.0 (trace)  | 1.10 |
|               | 0.68         | 1.50 |
|               | 0.96         | 1.20 |
|               | 1.30         | 0.70 |
|               | 2.00         | 0.82 |
|               | 1.47         | 0.68 |
|               | 1.47         | 1.50 |
|               | 0.00 (trace) | 1.65 |
|               | 0.00         | 0.00 |
|               | 0.75         | 0.70 |
|               | 0.40         | 0.50 |
|               | 2.10         | 1.50 |
|               | 0.85         | 0.50 |
|               | 0.75         | 0.80 |
|               | 0.00         | 0.40 |
| Average       |              | 0.90 |

in pounds, and the amount of galactose found in the urine during the five hours following ingestion.

It is seen in these two series containing sixty normal cases that the average urinary output of galactose is less than 1 gm. The highest output in the first series was 2.2 gm. and in my series, 2 gm.; while the great majority of normal persons excreted less than 1 gm.

From these observations it seems safe to state that an output of 3.0 gm. or more is abnormal. In my series it has been found invariably that in normal persons galactose, if excreted at all, appears in the first and second specimens and rarely is found in the later specimens.

At the Cleveland Clinic, galactose has been used as a test of liver function for over a year. The results of this investigation may be summarized as follows: From the standpoint of the amount of galactosuria, the patients tested fall into two general but rather definite groups. In those excreting *abnormal* amounts of galactose, the clinical impression pointed toward diffuse liver damage; in those with a *normal* output, clinical investigation or subsequent exploration revealed the jaundice to be due to biliary obstruction. A summary and comparison of clinical and operative findings with the results of the galactose tolerance test are presented. (Table IIA and IIB.)

In the first group of undoubted cases of biliary obstruction, the galactose test, prior to operation, showed in each of these a normal secretion, averaging 0.87 gm.

In the second group, however, in which the diagnosis of diffuse liver damage is reasonably certain, marked galactosuria ranging from 1.90 to 9.1 gm. (average over 5 gm.) was present. These results correspond very well with the observations of Shay, Schloss and Rodis.<sup>12</sup>

A review of the histories of several of these patients gives some impression of the value of the test in differential diagnosis.

CASE 1.—A man, aged fifty-four years, was admitted to the hospital, complaining of hunger pains, itching, and jaundice of one week's duration. The past history was irrelevant except that he had noticed a gradually increasing epigastric pain before meals during the preceding year. Physical examination revealed moderate, generalized icterus, fair nutrition, and an enlargement of the liver, which was described as being 6 cm. below the costal margin, rough, irregular, and not tender. The cholecystogram showed a nonfunctioning gallbladder. The bromsulphalein test showed 80 per cent retention of dye in one-half hour. The clinical diagnosis was "common duct obstruction, probably stone." The galactose test showed an output of 1.8 gm., corroborating the impression of obstruction. Operation revealed an inoperable cancer at the head of the pancreas.

CASE 2.—The patient, a man, aged forty-two years, was admitted to the clinic, complaining of indigestion, belching, progressively increasing jaundice, and the loss of 10 pounds in weight in the course of three weeks. The patient had had several phallic sores and a positive blood Wassermann two months before, for which he had had no treatment. The patient was well nourished, moderately jaundiced, with a slow pulse rate and slight tenderness over the right upper quadrant. The liver was palpable 3 cm. below the costal margin. The Wassermann test was weakly positive, the bromsulphalein test showed a retention of 30 per cent in half an hour and the galactose test an output of 3.3 gm.—confirming the clinical impression of catarrhal jaundice or syphilitic hepatitis.

CASE 3.—A housewife, aged twenty-six years, came to the clinic complaining of jaundice, weakness, and itching. Four years before, the gallbladder had been removed. Two years

TABLE II  
SUMMARY AND COMPARISON OF CLINICAL FINDINGS WITH THE GALACTOSE TOLERANCE TEST  
A. *Jaundice of the Obstructive Type*

| AGE | DIAGNOSIS              | BASIS FOR<br>DIAGNOSIS | DURATION OF<br>JAUNDICE | BROMSULPHIA-<br>LEIN TEST | VAN DEN BERGH<br>TEST | ICTERUS<br>INDEX | GALACTOSE SE-<br>CRETION (GM.) |
|-----|------------------------|------------------------|-------------------------|---------------------------|-----------------------|------------------|--------------------------------|
| 54  | Carcinoma—pancreas     | operation              | 6 weeks                 |                           | 12.5                  | 100              | 1.80                           |
| 45  | Carcinoma—pancreas     | operation              | ?                       |                           | indirect              | 50               | 1.47                           |
| 21  | Stricture—common duct  | operation              | 6 months*               |                           | 11.0                  |                  | 0.00                           |
| 58  | Stone—common duct      | operation              | 4 months*               |                           | 20.0                  | 60               | 0.50                           |
| 21  | Biliary fistula        | operation              | 2 months*               |                           | 16.0                  |                  | 0.50                           |
| 50  | Carcinoma—hepatic duct | operation              | 6 weeks                 |                           | 18.0                  | -                | 1.30                           |
| 26  | Stricture—common duct  | operation              | 8 months*               |                           |                       | 75               | 0.63                           |
| 55  | Stricture—common duct  | operation              |                         |                           |                       |                  | 0.85                           |
| 44  | Obstructive jaundice   | autopsy                | 5 days                  |                           | 12.8                  | 50               | 0.83                           |
| 54  | Stricture—common duct  | clinical<br>operation  | 6 months*               |                           | 2.7                   |                  | 0.96                           |
|     |                        |                        |                         |                           |                       | Average          | 0.87                           |

B. *Jaundice Due to Hepatitis or Hepatic Degeneration*

|    |                                            |           |                 |          |      |         |      |
|----|--------------------------------------------|-----------|-----------------|----------|------|---------|------|
| 56 | Hypertrophism                              | clinical  | ? weeks         | 100-60%  |      | 50      | 3.20 |
| 41 | Cirrhosis—ascites                          | clinical  | 2 weeks (?)     | 100-80%  | 6.8  | 100     | 6.00 |
| 53 | Cirrhosis—ascites                          | clinical  | ? weeks         | 100-90%  |      |         | 6.00 |
| 30 | Arsenic hepatitis                          | clinical  | 1 week          | 100-100% |      | 100     | 9.10 |
| 43 | Catarrhal jaundice or syphilitic hepatitis | clinical  | 3 weeks         | 100-30%  | 9.0  |         | 3.30 |
| 32 | Cirrhosis                                  | autopsy   | 6 weeks         | 80-50%   | 5.8  | 75      | 4.70 |
| 66 | Catarrhal jaundice                         | operation | 3 weeks         |          | 5.8  |         | 4.10 |
| 56 | Catarrhal jaundice                         | clinical  | 2 weeks         | 100-100% | 19.7 |         | 7.00 |
| 42 | Catarrhal jaundice                         | clinical  | 10 days         | 50-30%   |      |         | 3.90 |
| 70 | Cirrhosis(?) malignancy(?)                 | clinical  | 3 weeks         |          |      |         | 4.95 |
| 36 | Arsenic hepatitis                          | clinical* | 6 weeks (faint) |          |      |         | 1.90 |
| 50 | Cinephophen hepatitis                      | clinical  | 1 week          |          |      | 100     | 6.00 |
| 27 | Catarrhal jaundice                         | clinical  | 2 days          |          | 15.0 | 100     | 5.00 |
| 62 | Catarrhal jaundice                         | clinical  | 1 week          |          |      | 75      | 8.00 |
|    |                                            |           |                 |          |      | Average | 5.22 |

\*Intermittent.



later the patient had jaundice and was operated on again. At that time a small stone was removed from the common duct with subsequent drainage of bile for ten weeks after the operation. Eight months previous to her visit to the clinic, jaundice again had appeared in intermittent attacks with recurrent pain in the left upper quadrant, which pain had persisted. Examination revealed emaciation, generalized icterus, and a large tender liver, with questionable splenic enlargement. The patient had a slight fever, and a slow pulse rate.

The bromsulphalein test showed 60 per cent retention in one-half hour. The icterus index was 75. The clinical impression was that the jaundice was due either to common duct stone or to adhesions. The galactose test showed a normal excretion of 0.63 gm. At operation the common duct was found occluded by adhesions. The liver was normal.

CASE 4.—A woman, aged thirty-two years, was admitted to the hospital complaining of jaundice and weakness and with the story of having had nausea and vomiting, recurrent dull pain in the right upper quadrant, and progressive jaundice of three weeks' duration. Physical examination revealed a poorly nourished young woman with moderate icterus and tenderness over the right lobe of the liver. The cholecystogram showed a functioning gallbladder. The van den Bergh test was direct; the serum bilirubin was 5.8 gm. The clinical impression was "stone in the common duct." The galactose test showed an output of 4.1 gm.—indicating an intrahepatic process, rather than obstruction of the duct. Operation for stone did not disclose any obstruction. The appendix was removed, and the patient recovered uneventfully.

This is an excellent example of the value of the galactose test before operative procedures are considered too hastily.

CASE 5.—A dentist, fifty-six years of age, was admitted to the hospital complaining of weakness and slight indigestion for three months. Three weeks before admission he had begun to experience vague epigastric distress and pain in the right upper quadrant, some nausea and clay-colored stools and, ten days before admission, the development of a steadily deepening jaundice. The physical examination revealed generalized icterus, fair nutrition, normal temperature, and a large, tender liver. The gallbladder was nonfunctioning, according to the roentgenogram. The bromsulphalein test showed a retention of 100 per cent, the serum bilirubin was 20 mg., and the galactose output on two occasions was 7.0 and 6.5 gm., respectively. There was much debate as to the nature of jaundice in this case. Operation was seriously considered, but since the jaundice seemed to be subsiding, this was abandoned. The galactose test revealed that the cause of jaundice was intrahepatic, probably of catarrhal nature, and not obstructive.

In reviewing these five representative cases, it is evident that the ability or failure of the liver to metabolize the galactose has given valuable negative evidence as well as positive findings in cases in which operative interference has been considered.

From the standpoint of prognosis, the test also is of value. Several of the patients with catarrhal jaundice or toxic hepatitis have had subsequent galactose tests and concomitant with clinical improvement, and in several instances even before improvement was obvious clinically, the excretion of galactose gradually fell to normal. These patients all recovered. (Table III.)

It has been of interest to review in the literature the cases studied with this test. In his early work, Bauer thought that he had found a test for cirrhosis of the liver. Two years later he reported the finding of an alimentary galactosuria in catarrhal jaundice and described ten such cases showing galactosuria which gradually decreased as the patients improved.<sup>13</sup> He considered an output of more than 3 gm. as indicating a pathologic condition of the liver.

In the comparative series of cases recently published by Shay, Schloss and

TABLE III  
PROGRESS STUDIES OF GALACTOSURIA

| DIAGNOSIS            | DURATION<br>(DAYS) | GALACTOSE<br>EXCRETION<br>(GRAMS) | OUTCOME  |
|----------------------|--------------------|-----------------------------------|----------|
| Catarrhal jaundice   | 2                  | 5.0                               | Well     |
|                      | 5                  | 3.3                               |          |
|                      | 29                 | 0.4                               |          |
| Cinchophen hepatitis | 7                  | 6.0                               | Improved |
|                      | 11                 | 4.0                               |          |
|                      | 14                 | 4.2                               |          |
|                      | 17                 | 3.8                               |          |
|                      | 21                 | 3.0                               |          |
| Arsenic hepatitis    | 7                  | 9.1                               | Well     |
|                      | 21                 | 2.3                               |          |
| Catarrhal jaundice   | 14                 | 7.0                               | Well     |
|                      | 15                 | 6.5                               |          |
|                      | 21                 | 2.0                               |          |

Rodis, an output of galactose of more than 3 gm. was considered indicative of liver damage, and these authors expressed the thought that the element of chance may work against the test in both of these types of cases. For in mild catarrhal jaundice or hepatitis the carbohydrate metabolism may be only slightly altered and may tend to rapid recovery, while in the other group in which there is chronic damage to liver cells, destruction may progress to a state of diffuse involvement. It is also known that a so-called catarrhal jaundice or hepatitis may complicate the course of a chronic liver disease, such as cirrhosis.

In the series here reported, I have seen and listed several such instances of advanced cirrhosis with ascites and the terminal development of jaundice. In all of these cases, there was an abnormal excretion of galactose. In the average uncomplicated case of cirrhosis, however, assuming that only fibrosis is present, it is obvious that the carbohydrate metabolic function of the liver is normal and that only the permeability or excretory function is deranged. (Table IV.)

TABLE IV  
GALACTOSE EXCRETION IN FOCAL OR CHRONIC LIVER DISEASE

| AGE | DIAGNOSIS          | ASSOCIATED CONDITION        | BASIS FOR DIAGNOSIS             | BROM-SULPH-ALEIN | GALACTOSE EXCRETION |
|-----|--------------------|-----------------------------|---------------------------------|------------------|---------------------|
| 48  | Carcinoma stomach  | Metastases—liver            | Autopsy                         | 30-20%           | 1.78                |
| 63  | Cirrhosis          | Diabetes—ascites            | Clinical                        | 100-50%          | 3.82                |
| 62  | Cirrhosis          | Ascites                     | Autopsy                         | 100-80%          | 1.27                |
| 49  | Cirrhosis          | Hyperthyroidism             | Autopsy                         | -----            | 0.00                |
| ?   | Cirrhosis          | -----                       | Autopsy                         | -----            | trace               |
| 48  | Carcinoma—site (?) | Metastases—lung, peritoneum | Clinical                        | -----            | 0.82                |
|     | Nephrosis          | Edema                       | Clinical                        | -----            | 0.50                |
| 21  | Banti's            | ? Cirrhosis                 | Clinical and operation          | -----            | trace               |
| 50  | Cardiac            | ? Cirrhosis                 | Clinical                        | 60-20%           | 0.84                |
|     | Pernicious anemia  | Parkinsonism                | Clinical                        | -----            | trace               |
| 52  | Toxic hepatitis    | Jaundice (2 months)         | Operation<br>Pathologic section | 80-80%           | trace               |

To recapitulate—practically all of the cases which have been reported as showing an abnormal excretion of galactose fall into the group in which it is reasonable to believe that a relatively acute diffuse liver damage has taken place. Among these may be listed cases of catarrhal jaundice, arsphenamine hepatitis, phosphorous poisoning, acute yellow atrophy, syphilitic hepatitis, and the hepatitis of infection or intoxication, such as is occasionally seen in pneumonia or hyperthyroidism.

In conclusion, it seems that the galactose tolerance test of liver function furnishes a simple but valuable procedure which clearly identifies and differentiates the toxic or infectious type of jaundice from the jaundice due to extrahepatic causes.

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# LABORATORY METHODS

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## THE DETERMINATION OF SERUM PROTEIN BY MEASUREMENT OF THE VOLUME OF PRECIPITATE\*

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THE protein content of urine and cerebrospinal fluid is commonly determined by the measurement of the volume of protein precipitated by certain reagents. Shevky and Stafford<sup>1</sup> compared previous clinical precipitation methods for protein and developed a more accurate one, using Tsuehiya's reagent. This method has been used and modified by McNaught<sup>1</sup> for cerebrospinal fluid and by MacKay (Peters and Van Slyke<sup>2</sup>) for urine. Samson<sup>3</sup> has presented a micro-precipitation method for serum protein as well as for serum albumin and globulin but does not give figures for the absolute amounts of protein in either case or compare his values with those of other standard methods. In the hope of making available a simple clinical method for blood protein, comparative determinations have been made both by the precipitation and by macro-Kjeldahl methods.

### TECHNIC

The procedure adopted was that given for urine by MacKay with the exception that the volume of protein precipitated was compared with the volume of precipitate from a standard casein solution (McNaught). For this standard a solution containing 3.0 gm. casein, 1.0 gm. sodium bicarbonate, 25.0 gm. sodium chloride, and 20 c.c. formalin was made up to a liter and filtered. The nitrogen was then determined by the Kjeldahl method. Such a standard keeps indefinitely at room temperature. The serum was diluted 1 to 50 and 1 to 75 with 2.5 per cent sodium chloride. Four-cubic-centimeter amounts of diluted serum were put in Shevky-Stafford tubes, two tubes being used for the standard, two for the 1-to-50 dilution, and two for the 1-to-75 dilution. The volume of precipitate was thus brought into range with the volume from the standard. Tsuehiya's reagent (1.5 gm. phosphotungstic acid and 5 c.c. concentrated hydrochloric acid made up to 100 c.c. with 95 per cent alcohol) was added up to the 6.5 mark; the tubes were then inverted slowly three times and, after exactly ten minutes, centrifuged for ten minutes at 1,800 revolutions per minute.

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Calculation was made as follows:

$$\frac{\text{Vol. ppt. unknown}}{\text{Vol. ppt. standard}} \times \frac{\text{Grams \% protein in standard}}{\text{Dilution of unknown}} = \text{Grams \% protein in unknown serum}$$

## EXPERIMENTAL RESULTS

Results by the above method are shown in Table I.

The procedure used was adopted as the result of numerous attempts to make the precipitation method accurate for serum. It was found unsatisfactory merely to multiply the volume of precipitate by the dilution and by the factor 0.72, as is done to obtain the amount of protein in urine. Values for serum protein thus obtained vary with the dilution and with uncontrolled changes in the speed of the centrifuge. Therefore a standard protein solution was used. Various modifications, as the use of sodium tungstate precipitation, the use of different standards (horse serum, human serum, albumin, and casein as shown in Table II), a longer period of centrifuging and dilution of the serum and

TABLE I  
PRECIPITATION OF SERUM PROTEIN BY TSUCHIYA'S REAGENT

| SERUM ANALYZED                                                      | SERUM<br>C.C.<br>PPT.* | STAND.<br>C.C.<br>PPT.* | GM. %<br>PROT. IN<br>CASEIN<br>STANDARD | DILU-<br>TION<br>OF<br>SERUM | GM. %<br>PROTEIN | GM. %<br>PROTEIN<br>(KJELDAHL) | PER-<br>CENTAGE<br>OF<br>ERROR |
|---------------------------------------------------------------------|------------------------|-------------------------|-----------------------------------------|------------------------------|------------------|--------------------------------|--------------------------------|
| 1. Normal human serum.                                              | 0.128                  | 0.126                   | 0.0929                                  | 1:75                         | 7.08             | 7.08                           | 0                              |
|                                                                     | 0.120                  | 0.121                   | 0.0929                                  | 1:75                         | 6.91             | 7.08                           | - 2.4                          |
|                                                                     | 0.122                  | 0.120                   | 0.0929                                  | 1:75                         | 7.08             | 7.08                           | 0                              |
| 2. Normal human serum.                                              | 0.160                  | 0.162                   | 0.1394                                  | 1:50                         | 6.88             | 7.06                           | - 2.5                          |
|                                                                     | 0.133                  | 0.129                   | 0.0929                                  | 1:74.3                       | 7.11             | 7.06                           | + 0.7                          |
|                                                                     | 0.129                  | 0.129                   | 0.0929                                  | 1:75                         | 6.96             | 7.06                           | - 1.4                          |
|                                                                     | 0.114                  | 0.108                   | 0.0929                                  | 1:75                         | 7.35             | 7.06                           | + 4.1                          |
|                                                                     | 0.115                  | 0.114                   | 0.0929                                  | 1:75                         | 7.02             | 7.06                           | - 0.6                          |
| 3. Normal human serum.                                              | 0.179                  | 0.205                   | 0.1567                                  | 1:50                         | 6.85             | 6.78                           | + 1.0                          |
|                                                                     | 0.186                  | 0.200                   | 0.1567                                  | 1:50                         | 6.49             | 6.78                           | - 4.3                          |
|                                                                     | 0.128                  | 0.148                   | 0.0929                                  | 1:75                         | 6.03             | 6.78                           | -11.1                          |
|                                                                     | 0.131                  | 0.147                   | 0.0929                                  | 1:75                         | 6.21             | 6.78                           | - 8.4                          |
| 4. Aortic regurgitation.<br>Syphilis.                               | 0.108                  | 0.105                   | 0.0929                                  | 1:75                         | 7.18             | 7.52                           | - 4.5                          |
|                                                                     | 0.117                  | 0.119                   | 0.0943                                  | 1:75                         | 6.95             | 7.52                           | - 7.6                          |
| 5. Malignant hyperten-<br>sion. Cardiac asthma.                     | 0.102                  | 0.109                   | 0.0943                                  | 1:75                         | 6.61             | 6.69                           | - 1.2                          |
| 6. Hypertension.<br>Syphilitic heart dis-<br>ease. Nephrosclerosis. | 0.131                  | 0.106                   | 0.0943                                  | 1:50                         | 5.82             | 6.44                           | - 9.6                          |
| 7. Hypertension.<br>Arteriosclerosis.                               | 0.100                  | 0.099                   | 0.0848                                  | 1:75                         | 6.42             | 6.41                           | + 0.2                          |
|                                                                     | 0.100                  | 0.108                   | 0.0848                                  | 1:75                         | 5.89             | 6.41                           | - 8.1                          |
|                                                                     | 0.105                  | 0.105                   | 0.0848                                  | 1:74.6                       | 6.32             | 6.41                           | - 1.4                          |
| 8. Arteriosclerotic heart<br>disease with decom-<br>pensation.      | 0.135                  | 0.130                   | 0.0943                                  | 1:50                         | 4.89             | 5.81                           | - 5.6                          |
| 9. Arteriosclerotic heart<br>disease. Nephro-<br>sclerosis.         | 0.115                  | 0.125                   | 0.0943                                  | 1:50                         | 4.34             | 4.84                           | -10.3                          |
|                                                                     | 0.123                  | 0.132                   | 0.0943                                  | 1:50                         | 4.39             | 4.84                           | - 9.3                          |
| 10. Glomerular nephritis.<br>Pulmonary congestion.                  | 0.095                  | 0.110                   | 0.0943                                  | 1:50                         | 4.07             | 4.47                           | - 8.9                          |

\*Each figure is the average from duplicate tubes.

TABLE II  
USE OF DIFFERENT STANDARDS IN THE PRECIPITATION OF SERUM PROTEIN  
BY TSUCHIYA'S REAGENT

| SERUM ANALYZED                                       | STANDARD USED  | GM. %<br>PROTEIN | GM. %<br>PROTEIN<br>(KJEL.) | PER-<br>CENTAGE<br>OF<br>ERROR |
|------------------------------------------------------|----------------|------------------|-----------------------------|--------------------------------|
| Normal human serum I                                 | Horse serum A  | 6.94             | 7.24                        | - 4.1                          |
|                                                      | Horse serum A  | 7.46             | 7.24                        | + 3.1                          |
|                                                      | Human serum II | 6.58             | 7.24                        | - 9.1                          |
|                                                      | Casein A       | 6.82             | 7.24                        | - 5.8                          |
|                                                      | Casein A       | 6.92             | 7.24                        | - 4.4                          |
| Normal human serum II                                | Human serum I  | 7.24             | 6.92                        | + 4.6                          |
|                                                      | Human serum I  | 7.01             | 6.92                        | + 1.3                          |
| Normal human serum III                               | Human serum IV | 7.58             | 7.61                        | - 0.4                          |
|                                                      | Human serum IV | 7.84             | 7.61                        | + 3.2                          |
| From patient with multiple sclerosis and<br>epilepsy | Casein B       | 6.69             | 6.87                        | - 2.6                          |
|                                                      | Casein B       | 7.01             | 6.87                        | + 2.0                          |
|                                                      | Albumin A      | 7.39             | 6.87                        | + 7.6                          |
| From patient with syphilitic meningitis              | Human serum IV | 6.87             | 6.65                        | + 3.3                          |
|                                                      | Human serum IV | 7.00             | 6.65                        | + 5.3                          |
|                                                      | Casein B       | 6.87             | 6.65                        | + 3.3                          |
|                                                      | Casein B       | 5.81             | 6.65                        | -12.6                          |
|                                                      | Albumin A      | 6.97             | 6.65                        | + 4.8                          |
| From patient with chronic edema                      | Albumin A      | 6.71             | 6.65                        | + 0.9                          |
|                                                      | Casein B       | 3.68             | 3.85                        | - 4.4                          |
|                                                      | Casein B       | 3.78             | 3.85                        | - 1.8                          |
|                                                      | Albumin A      | 3.68             | 3.85                        | - 4.4                          |

standard with different concentrations of saline have been tried in attempts to make the method more accurate. Results with different concentrations of saline are shown in Table III.

TABLE III

| CONCENTRATION OF SALINE | AVERAGE PERCENTAGE ERROR |
|-------------------------|--------------------------|
| 0.8%                    | +6.5                     |
| 1.0%                    | +4.0                     |
| 1.5%                    | +4.1                     |
| 2.5%                    | -4.0                     |

In a few cases the precipitates were resuspended in 80 per cent alcohol and recentrifuged. Considerable variation in the volumes of precipitate in duplicate tubes was found in all of these modifications. For example, five volumes of precipitate from the same solution, centrifuged at the same time, varied in one case from 0.175 to 0.190 c.c. of precipitate. In the later determinations it was thus considered necessary to compare duplicate tubes on all samples.

From the tables it may be seen that the majority of the results have an error of less than 5 per cent although they may occasionally vary from the Kjeldahl determination by as much as 12 per cent, especially when the amount of protein is small.

The average percentage error (+6.5 to -4.0 per cent) compares favorably with the error found by Shevky and Stafford<sup>4</sup> for urine (average error, 8.4 per cent; standard deviation,  $\pm 9.7$  per cent). The results obtained by McNaught<sup>1</sup> on cerebrospinal fluid are, however, somewhat more accurate (1.5 to 5.1 per cent error). It is recognized that an inherent error exists in methods based on the precipitation of colloids, the physical state of which may vary in different ex-

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periments. Factors which affect such precipitation have been discussed by Samson.<sup>3</sup> Of these, the ones which have not been controlled in the present determinations are the size and specific gravity of the particles precipitated, the specific gravity and viscosity of the fluid in which precipitation takes place, and to some extent the temperature of the system.

## SUMMARY

A simple method for the determination of serum protein is presented and discussed. The volume of protein precipitated from diluted serum by a photungstic acid reagent has been measured in Shevsky-Stafford tubes and compared with the volume from a standard protein solution.

With the proper standard at hand, the simplicity and speed of the precipitation method and the small amount of equipment needed, as compared with the Kjeldahl determination, make the precipitation method applicable for clinical use.

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## THE REFINING OF ANTISERUMS\*

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### INTRODUCTION

THE method of refining and concentrating antiserums and antiplasma which is used in this laboratory will be described in this paper. This method is being used for antipneumococcus and antimeningococcus serums and in modified form for other serums. The experimental procedure will first be outlined; then modifications and explanations of the procedure will be given; and finally a brief discussion and summary of the relations will be presented.

### EXPERIMENTAL PROCEDURE

The method to be described is that used primarily for refining pneumococcus antiserums.

a. *Preparation of Serum.*†—Immediately after the separation of the serum, 0.5 per cent phenol was added in the form of a solution containing five parts phenol and six parts ether.<sup>1</sup> If this immediate addition was not possible, the phenol was added as soon as practicable. The serum was kept cold. Before beginning the further treatment, the serum was tested as to sterility. If bacteria were present, the serum was filtered through a Berkefeld filter, No. N.

b. *Dialysis of Serum.*—The serum, containing 0.5 per cent phenol, was well mixed, placed in cellophane (No. 600) bags, from 3 to 4 liters in each. To each bag 10 c.c. toluene and 5 c.c. chloroform were added. Each bag was then tied tightly near the top, the top turned over and tied, or an additional piece of cellophane placed over it, in order to eliminate the possibility of contaminants dropping into the bag. Dialysis against running tap water was allowed to proceed for from five to seven days. After forty-eight hours and again after ninety-six hours, 0.2 per cent phenol (in ether) was added to each bag. The temperature of the tap water ranged from 12° to 25° C. at different times of the year. The duration of the dialysis was greater with the cooler water. The sterility of the dialysate was tested after dialysis.

c. *"Acid Protein" Precipitation.*—The dialysates were removed from the bags and mixed, the bags washed with several hundred cubic centimeters of distilled water, which were added to the main bulk. Dry sodium chloride to bring the solution to N/20 was added (2.92 g. per liter). The solution was placed in the ice box overnight. Early the next morning, it was brought to  $P_H$  5.1 with normal acetic acid ( $\gamma$  - dinitrophenol as indicator), allowed to stand in the

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†The serum was obtained from horses immunized with the centrifuged sediment of pneumococcus broth cultures taken up with saline and the vaccine killed with formalin. In general terms, the horses were inoculated three successive days, rested six days, and the series repeated with increasing dosages up to a certain maximum. Test bleedings were taken every two weeks.



cold for from four to five hours, and filtered through paper pulp and "super cel"<sup>2</sup> on a Buchner funnel until clear.

d. *Preliminary Tests for Antibacterial Substance Precipitation.*—The filtered solution was brought to  $P_H$  5.9 with N NaOH and kept in the ice box. Portions of 50 c.c. were brought to  $P_H$  5.9, 6.1, 6.3, and 6.8 (with N NaOH where necessary) (p-nitrophenol as indicator), diluted with from four to five volumes of Berkefeld filtered tap water, and allowed to stand in the ice box overnight. The next day the mixtures were centrifuged, the precipitates dissolved in one-fifth the volume (10 c.c. each) of 1 per cent sodium chloride solution, and the supernatants were tested for antibacterial substance contents by precipitin or agglutinin reactions (or both.)<sup>2</sup> In estimating the contents in this way, account was, of course, taken of the concentration or dilution of the antibacterial substance in comparison with the original serum.

These tests were carried out under the direction of Miss Georgia Cooper, who outlined the procedure as follows:

"The titrations were made with quantities of antiserum from the following range, which were selected to show the character and the end point of the reaction; 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, 0.0005, 0.0002, 0.00001, 0.00005 c.c.

"For the agglutination tests the antisera were so diluted with physiologic saline that the requisite amounts were contained in 0.5 c.c. Five-tenths cubic centimeter of each of the dilutions and 0.5 c.c. of a fresh eighteen- to twenty-hour broth culture of pneumococci were mixed in test tubes and incubated for two hours at 55° C. Readings were made. The tubes were stored in the ice box overnight and readings again made.

"For the precipitation tests 0.1, 0.05, and 0.02 c.c. of the undiluted antisera and the smaller quantities diluted to be contained in 0.05 c.c. were obtained with 0.1 c.c. of the precipitating antigens in test tubes. The tubes were rolled for two minutes. Five-tenths cubic centimeter saline solution was then added to each tube and readings were made. The precipitation antigen was prepared by growing pneumococci in phosphate beef-heart broth for about a week, at the end of which the organisms generally were found to be autolyzed. The precipitation antigens before use, when necessary, were centrifuged until clear. With certain exceptions, the end points of precipitation, and of agglutination to a lesser degree, indicated with a moderate degree of accuracy the relative amounts of mouse protective antibacterial substances in the antisera tested."

If the supernatants showed the presence of appreciable quantities of antibacterial substance (10 per cent or more of the original), in place of procedures "e" and "f" which follow, recourse must be had to procedures "e1" and "f1."

e. *Precipitation of Antibacterial Substance.*—The solution was brought to the desired  $P_H$  as found in the preliminary tests, four volumes of Berkefeld filtered tap water containing 0.2 per cent phenol added, and placed in the ice box overnight. The precipitate in general settled well. The bulk of the supernatant was siphoned off, and the rest of the liquid separated by centrifuging.

<sup>2</sup>Obtained from the Johns-Manville Corporation, New York City.

f. *Solution of Antibacterial Substance.*—The precipitate was dissolved in from one-fifth to one-tenth of the original volume of 0.5 per cent sodium chloride solution containing 0.5 per cent phenol. The amount of solvent was governed in part by the potency of the original material and the potency desired in the final product, and in part by the physical consistency (viscosity) of the material after solution. The solution was placed in the ice box for at least twenty-four hours, then filtered through super cel and paper pulp to remove separated fibrin. One per cent additional sodium chloride (solid) was added and the solution stored at low temperature.

g. *Preparation of Antibacterial Solution for Clinical Use.*—The cold solution was allowed to warm to room temperature and filtered on a Buchner funnel through paper pulp and super cel and then through a Berkefeld filter. If filtration was slow through a fine Berkefeld, preliminary filtration through a coarser (V), followed by filtration through the finer (N), was used. The filtrate was tested for sterility and then bottled in 10, 15, or 25 c.c. vials, as desired. Suitable sterility tests on the vial contents were also carried out. The preparations were kept in the ice box. At times, solid material (presumably protein) separated in the vials at the low temperature but dissolved readily again at room temperature. Since the material is warmed at 37° C. before use clinically, this separation occasions no difficulty. Stock was labelled to be returned in six months. If the solutions became cloudy, they were refiltered and rebottled, as well as retested for potency. Potency tests, either mouse or agglutination and precipitation, were carried out as described elsewhere both on the filtered bulk and on the bottled materials.

e1. *Alternative Method of Precipitation of Antibacterial Substance.*—If the supernatants of the preliminary precipitation tests "d" showed appreciable quantities (10 per cent or more) of the original antibacterial substance, the following method was used. An equal volume of saturated ammonium sulphate solution was added with thorough stirring (so-called 50 per cent saturation), allowed to stand from four to five hours, and filtered on a number of large fluted papers. This filtration was complete overnight.

f1. *Solution of Antibacterial Substance.*—The precipitate was removed from the filters, pressed between sheets of filter paper in a press for some hours, placed in a cellophane bag containing 10 c.c. chloroform, and suitably tied and dialyzed against running tap water for from three to four days. To the solution after removal from the bag, 1.5 per cent sodium chloride and 0.5 per cent phenol (as phenol-ether mixture) were added. The volume was then from one-fourth to one-half of the volume before precipitation. The solution was placed in the ice box for at least a day and then filtered through paper pulp and super cel to remove separated fibrin. Further treatment followed that described in section "g."

#### NOTES ON THE EXPERIMENTAL PROCEDURE

A. The method outlined is given as applicable to antisera. Essentially the same procedure can be used with plasma. The greater amounts of fibrin (and fibrinogen) in the latter may increase the acid, perhaps other precipitates, and may also make filtration slower and more difficult. An essential feature of

the method consists in keeping the material as free from bacterial growth as possible. The importance of this fact and the desirability of having no products of bacterial growth present in the final products was recognized early and strongly emphasized, among others by Anderson<sup>2</sup> in 1909 and by Avery and his coworkers<sup>3</sup> in 1917.

B. Dialysis of the serum was an essential feature of the Banzhaf-Klein procedure.<sup>4</sup> This procedure was stated to depend upon the observations of Avery<sup>5</sup> and of Felton<sup>6</sup> that pneumococcus antibacterial substances were precipitated from serums by dilution with distilled water. Dialysis in the Banzhaf-Klein procedure was assumed to remove the salts and so play the part that was taken by dilution. It may be stated that, as a result of dialysis and the accompanying removal of salts, partial, but not complete precipitation of antibacterial substance occurred. The function of the dialysis did not, therefore, appear to be altogether simple. In addition to removing the salts, it evidently involved changes in the character of the globulins. Such changes have been described by Svedberg,<sup>7,8</sup> who, as a result of molecular weight determinations, considered that one globulin was present in serum initially and that different treatments broke it down into fractions designated pseudoglobulin and euglobulin, and by Reiner and Reiner,<sup>9</sup> who showed that repeated precipitations resolved a globulin precipitable at a definite  $P_H$  value (from 5.5 to 6.0), into two globulins precipitable at two different  $P_H$  values (5.1 and 6.7). Dialysis evidently caused changes in the natures of the protein fractions which resulted in the subsequent procedures furnishing an immunologically useful product. It was found possible to keep the contents of the bags sterile throughout the dialysis by using the precautions given, although tests<sup>6</sup> showed that about 70 per cent of the phenol dialyzed out of the bags within the first three days. If the cellophane bags were used for more than one dialysis, they were washed thoroughly and kept in 5 per cent phenol solution until used again.

C. The "acid protein" precipitation as developed by Felton<sup>10,11</sup> was an essential part of the method. With Types I and II pneumococcus antisera it was permissible to allow the precipitate in the solution to stand overnight in the ice box; with other antisera the time should be limited to five hours because of possible inactivation due to the acid condition.

D. The preliminary tests for the best conditions for the precipitation of antibacterial substance were very essential. It was naturally desirable to obtain these conditions in as short a time as possible. Agglutinin and precipitin tests were used, therefore, and not mouse protection potencies. Comparative results were sufficient for the purposes in view, namely, possible losses of material as shown in the tests on the supernatants. A large number of pneumococcus (as well as meningococcus and other) antisera were tested, but no regularities observed as to the most suitable hydrogen ion concentration. The two best values were found to be  $P_H$  5.9 - 6.1 and  $P_H$  6.8. Apparently a number of variables, including the organism and method used in inoculating the horses, the condition and state of the horses (whether they had been bled over a long or short period of time), the keeping and handling of the serums, etc., all played a part

\*Carried out by Edward Buxbaum.

in determining the properties of the protein substances present. These conditions, at present, are not controllable. In this connection, attention may be directed again to the work of Reiner and Reiner referred to earlier in this paper. The classification of proteins to be applied to these different precipitates is not altogether clear. The "acid precipitate" at  $P_H$  5.1, is in part fibrin and possibly euglobulin; the antibacterial substance precipitate may be considered to be pseudoglobulin, since practically all anti substances have been found to be associated with this protein fraction. However, this precipitate did not include all of the pseudoglobulin present in the serum. At times, antibacterial substance was found in the supernatant from such a precipitate. In such a case, recourse was had to precipitation with 50 per cent saturation of ammonium sulphate, the customary method of separating pseudoglobulin.

With the pneumococcus antisera, it was found a number of times that Types V and VII showed considerable quantities of antibacterial substance in the supernatants. However, this was not always typical of these types, but more probably due to the previous history of the horses in these special cases because sera of these types from different horses caused no difficulty.

The usual methods of carrying out the agglutination and precipitation tests were used. When practicable, both were employed.

E. In this manipulation it was evidently impossible to avoid contamination. Using 0.2 per cent phenol and working in the cold, it was found that bacterial growth was practically negligible or very small and the formation of appreciable quantities of products of such growth avoided.

F. With some preparations, after solution, a gradual separation of protein substance occurred. This may have been fibrin or a conversion product of the globulin. Before use, this cloud or suspension was removed. As a rule, it contained no appreciable amount of active material.

G. The question of sterility was predominately important here. The comparatively large concentration of protein present in the final product and the occasional difficulty of filtering through a Berkefeld filter, necessitated special care for this part of the procedure. After various series of tests, it was decided to use 0.5 per cent phenol in all cases although recent experiments, as yet unpublished, by Miss Rosenstein and her coworkers in this laboratory have thrown considerable light on the efficacy of various disinfectants with different contaminants. Because of the possibility of the solutions becoming permanently cloudy on standing, a time limit of six months was placed on the clinical use of the materials. If, upon expiration of this time, the solutions were clear and showed no marked decrease in potency, they could be used directly. If, however, a cloud or suspension was present which did not dissolve on warming to  $37^{\circ} C.$ , the preparation was pooled, refiltered, rebottled, and retested for sterility and potency.

Potency tests have been described and discussed in detail at various times. Agglutinin and precipitin tests were carried out by Miss Cooper essentially as described in "d" and "e." Mouse protection tests were also carried out under the direction and supervision of Miss Cooper. The details of the method were given at various times by Felton,<sup>10, 11</sup> Park and Cooper,<sup>12, 13</sup> Falk, McGuire,

Valentine and Whitney,<sup>14</sup> and perhaps in most satisfactory form by Beard and Clapp.<sup>15</sup>

E1. The necessity of precipitating the total pseudoglobulin fraction by 50 per cent saturation with ammonium sulphate was obvious. Because of the possible inactivation by the salt, the precipitate was filtered on the day it was formed.

F1. The danger of contamination was present in this part of the procedure. The presence of the chloroform, care in closing the cellophane bag, and rapid working when the material was exposed, however, limited bacterial growth.

#### DISCUSSION

The procedure described is essentially that proposed by Banzhaf and Klein,<sup>4</sup> but with a number of modifications. The "acid protein" precipitation developed by Felton<sup>10, 11</sup> is one of the most important single steps in the method. If properly carried out, this step is fairly dependable in removing chill-producing substances from the preparations. Another important feature of the method is the prevention of bacterial growth and the formation of products of such growth which, if present, may well accompany the antibacterial substances in the various manipulations and produce undesirable clinical reactions.

The method as described was developed with antibacterial substances obtained by the use of the different types of pneumococcus. It has, however, been used in the refining of other antisera as will be outlined presently. The method has been in use for over two years at the Laboratories of the Department of Health with generally satisfactory results as far as the evidence available indicates. This does not mean that methods used in other laboratories such as various salt precipitations, or the alcohol method of Felton<sup>16</sup> are not equally satisfactory. The method described here is preferred by the writers because of its comparative simplicity.

The chill-producing and possibly other deleterious substances which are occasionally present offer the most direct problem for study. Felton's "acid protein" precipitation method is the most useful process available for eliminating such substances. With some sera one refinement is not sufficient to remove all of these substances, and the process must be repeated (including dialysis for several days, etc.). It is evident that where large amounts of antiserum, such as for pneumococcus Types I and II, are available, it is possible to carry out two, or even more, refinements with profit. With the other pneumococcus types where much less antiserum is available, the losses in refining comparatively small amounts, while in absolute quantity perhaps much the same as with large amounts, are so much greater in relative or percentage figures, that more than one refinement is inadvisable. This is perhaps the reason for the somewhat greater number of reactions observed clinically with the less common types of pneumococcus antiserum preparations, and not the fact that one process of refining is better than another, or that more information is available about certain types.

At the same time, much remains to be done in studying antisera for the various types. For example, as stated before, in carrying out this work, it was

found that certain preparations of Type V and Type VII pneumococcus antisera could not be refined by the simple dilution procedure, but required ammonium sulphate for precipitation. Similar materials from different horses could, however, be so refined. The cause for the different behavior is, at present, unknown. All that can be said is that the antibacterial substances were present in different fractions of the "pseudoglobulin" in these cases and that they existed in all probability in different fractions of the protein material in the sera of the horses. Such differences may well be due to repeated and long-continued inoculations and bleedings of the horses.

In connection with the method described in this paper, several facts may be pointed out. The conditions under which the experiments were performed were not satisfactory so far as the possibility of maintaining sterility was concerned. Even under these unavoidably adverse conditions, it was possible in most of the experiments to maintain sterility in the dialyzed solutions. The chill reactions of some of the final products may have been caused by the bacterial contaminations and growths which took place in some of the refinings. But the fact may be stated with some degree of emphasis that, in the opinion of the writers, it is not only possible but feasible to maintain sterility, or at worst to inhibit bacterial growth, under suitable conditions of working. It may be stated again that the method of refining and concentrating antisera here described is in no sense complete and final. It marks a step in that a method is presented, simple to carry out, and generally satisfactory although the preparations may sometimes require a second or even third reworking.

The questions of determining potencies and the clinical efficacies of the refined preparations do not come within the scope of this paper except in so far as the satisfactory attainment of certain standards is a necessary condition for the use of the method. The study of potency by mouse tests has been given by various workers a number of times. Where sufficiently virulent cultures were available, mouse units were used; otherwise agglutination and precipitation tests were resorted to as measures of the degree of concentration of the antibacterial substances in the refining process. Obviously the final volume of a preparation as compared to the initial volume controls the degree of the concentration but it may be said in general terms that the antibacterial substances were concentrated between five and ten times in the final preparation. The losses in active materials were difficult to estimate because of the inaccuracies inherent in the experimental method of determining potencies. In order to give a figure, which, however, cannot be considered as of a high order of accuracy, it may be said that, as a rule, between 50 and 100 per cent of the active material was recovered.

The description of the method referred to pneumococcus antisera. The same method, with practically no changes, was used with meningococcus antisera with equal success. It may also be used for additional antibacterial substances. Its possible application to other materials may be of interest. For instance, some experiments were carried out with scarlet fever antiserum which contained both antitoxin and antibacterial substances. Following dialysis and "acid protein" precipitation, the antibacterial substances were separated by

dilution at the most suitable hydrogen ion concentration followed by the precipitation of the antitoxin with ammonium sulphate to 50 per cent saturation. The clinical results with these two preparations (or more properly, protein fractions) were too few to warrant any statement, but the method is suggestive and may prove of interest and value.

The work described treats of the practical features of refining and concentrating antibacterial substances. The more purely scientific aspects of the problem have not been considered. There is, in fact, no evidence as to the nature of the active groupings or constituents of the anti substances. It would seem as if the time were ripe for a more intensive study of the chemical natures of these physiologically active materials. They, including both antitoxins and antibacterial substances, belong to the protein group of pseudoglobulins, which in general terms are less soluble than albumins and more soluble than euglobulins. This classification of the proteins is still arbitrary and based upon differences in solubility and not, as far as known, upon definite differences in chemical compositions and structures. The work of Svedberg and of the Reiners, to which reference has been made, brings out the arbitrary character of the classifications. The change in solubility of certain constituents on treatments in refining indicates this fact. For example, heating diphtheria and tetanus antisera with ammonium sulphate to 30 per cent saturation for an hour at 58° C. converts some of the inactive pseudoglobulin into insoluble protein.<sup>17, 18</sup> On standing (or on dialysis), precipitates may be formed with antibacterial products. These phenomena appear to involve the conversion of "pseudoglobulin" into "euglobulin." However, when the arbitrary nature of the classification is considered, to speak of the conversion of such constituents means nothing. Part of a protein fraction on simple treatment becomes insoluble (perhaps in a different nomenclature, denatured), and that is all that is known. Such simple changes taken in connection with the physiologic actions, however, may offer a promising field for study. Similarly, the precipitation of antibacterial substances, best at fairly definite hydrogen ion concentrations, these concentrations differing somewhat with different preparations, would also repay study. So far, chemical analyses have not succeeded in throwing light on any of these phenomena.

#### SUMMARY

The methods in use at the Bureau of Laboratories of the Department of Health of New York City for refining bacterial antisera are described. Various applications of these methods are discussed and some general theoretical relations given.

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## QUANTITATIVE DETERMINATION OF CHLORAL HYDRATE IN BLOOD AND URINE\*

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GETTLER and Blume<sup>1</sup> have described a method based on the work of Cole<sup>2</sup> for the quantitative determination of small amounts of chloroform in tissues. The final step in the method is a pyridine color reaction with chloroform, known as the Fujiwara<sup>3</sup> color reaction. This color change is produced not only by chloroform but by all R-C-halogen<sub>3</sub> compounds.

In the course of some work we were doing on anesthetics, we found it necessary to determine the quantity of chloral hydrate in the blood and urine, and accordingly, after consultation with Dr. Gettler, we employed his method for this purpose. We met with difficulties, but these were overcome by certain modifications in the method. With these, we were able to determine quantitatively amounts of chloral in solutions containing as little as 0.001 per cent.

The procedure we employ for determining the amount of chloral hydrate

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in blood is as follows: In a thin-walled test tube, about 200 by 20 mm. in size, is placed 2 c.c. of a 40 per cent NaOH solution, to which is added 1 c.c. pyridine (colorless) and then 4 c.c. blood filtrate prepared by the Folin-Wu method. In a similar tube is placed the NaOH and pyridine and 4 c.c. of a solution of chloral hydrate, for a standard. In our work the standard chloral hydrate solutions ranged from 1:20,000 to 1:100,000, the solution finally used for each determination being approximately that corresponding in chloral content to the blood filtrate. The two tubes are shaken simultaneously and equally and kept in a boiling water-bath for exactly one minute. They are then cooled by placing them in an ice bath for one minute. Six cubic centimeters of water is then added to each and the contents mixed by inversion. The contents are then filtered into colorimeter cups and readings made in the usual manner. The readings should be made within ten minutes from the time of heating, as the color changes on standing longer.

For determining the chloral in the urine the same procedure is followed but here the urinary pigments may interfere with the colorimetric reading. To obviate this instead of the final dilution with 6 c.c. of water, part of the water is replaced by an amount of urine corresponding to that taken for the chloral determination. Since the pyridine reaction does not occur in the cold, samples from the same urine are used in the water replacement. It is advisable that the concentration of the unknown should range between 0.01 and 0.05 mg. per c.c. If it is below 0.01 mg. it is necessary to add known quantities of chloral to obtain a satisfactory reading.

In checking the accuracy of the method described for blood chloral concentration, quantities of chloral ranging from 0.25 to 1 mg. were added to 3 c.c. of blood and the color read against standard aqueous solutions. The blood figures were within  $\pm 3$  per cent of the standard.

It should be pointed out that the method used seems to apply only to free chloral, or at most chloral which has been very little modified. When conjugated with glucuronic acid, chloral does not develop the pyridine color.

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# A METHOD OF DAMPING THE KROGH TYPE OF SPIROMETER FOR STUDYING THE RESPIRATION OF RABBITS\*

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WHILE studying the effects of morphine and its derivatives on the respiration of rabbits the author had reason to believe that the Krogh spirometer being used did not follow accurately the respiratory volumes of the animals. The apparatus consisted of a 40-liter rebreathing tank connected directly to a

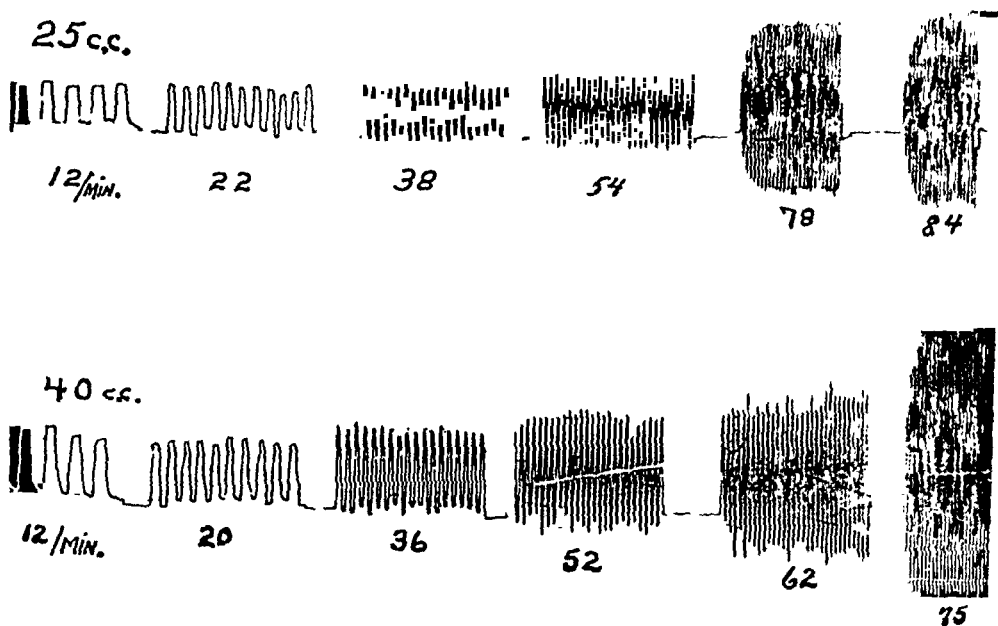


Fig. 1.—A record of the variations in amplitude of excursion of the undamped Krogh spirometer as described in the text. Upper tracing shows the variation at a volume of 25 c.c. and the rates (per minute) indicated. The lower tracing was made with a volume of 40 c.c. at the rates indicated.

Krogh spirometer (500 c.c. capacity) that recorded over two pulleys on a smoked kymograph paper. The float of the spirometer was made of phosphor bronze (0.038 cm. thick), and required a counterbalance weight of 80 gm. at a distance of 10 cm. from the axis of rotation. As a test of the ability of such a system to follow the respiratory volumes encountered, a 50 c.c. syringe was used to introduce known volumes of air into and out of the rebreathing tank. A kymograph record of the excursions of the spirometer showed at once that

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overshooting occurred at high rates (Fig. 1). The extent of this overshoot is shown by plotting the millimeter excursion of the spirometer against the rate at different volumes as given in Fig. 2. This graph shows an enormous overshoot starting at about 30 respirations per minute and a slight undershoot at rates below this point. It is obvious that with such a recording system no accurate

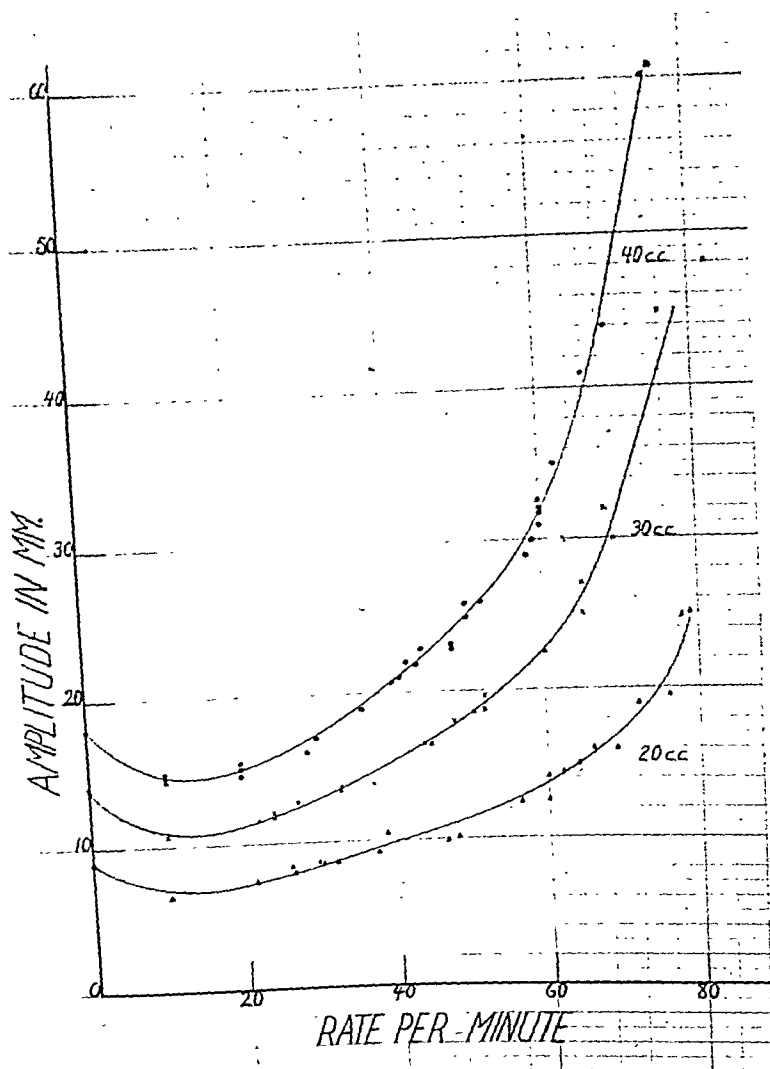


Fig. 2.—The variation in amplitude of excursion of the undamped Krogh spirometer with rate. Each curve represents a constant volume as indicated.

determination of respiratory volume can be made on rabbits since the respiratory rate of these animals varies between 30 and 80 per minute. Especially is this true if the animal is forced to breathe  $\text{CO}_2$ , which is frequently used as a respiratory stimulant.

A spirometer float of extremely thin (0.035 cm.) aluminum was made in an effort to reduce the mass, and thus the moment of inertia, sufficiently to allow

accurate recording at the rates involved. The overshoot was reduced, but by no means eliminated.

Finally an aluminum damping vane (diameter 5 cm.) was fastened, by

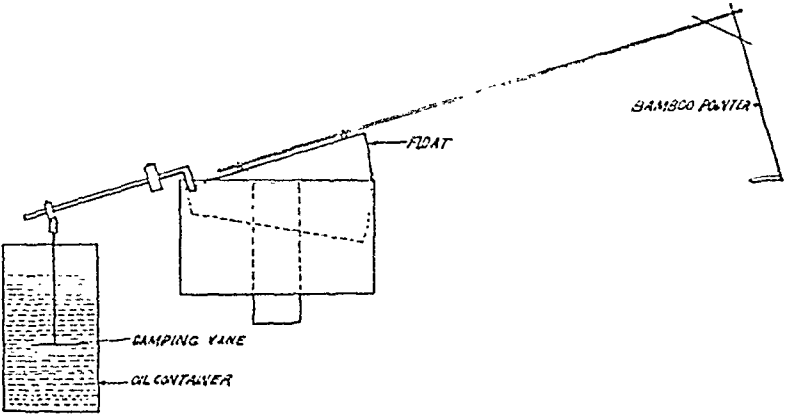


Fig. 3.—Diagram of the Krogh spirometer and the method of damping. The float, counterbalance arm, damping vane, and attachment are made of aluminum.

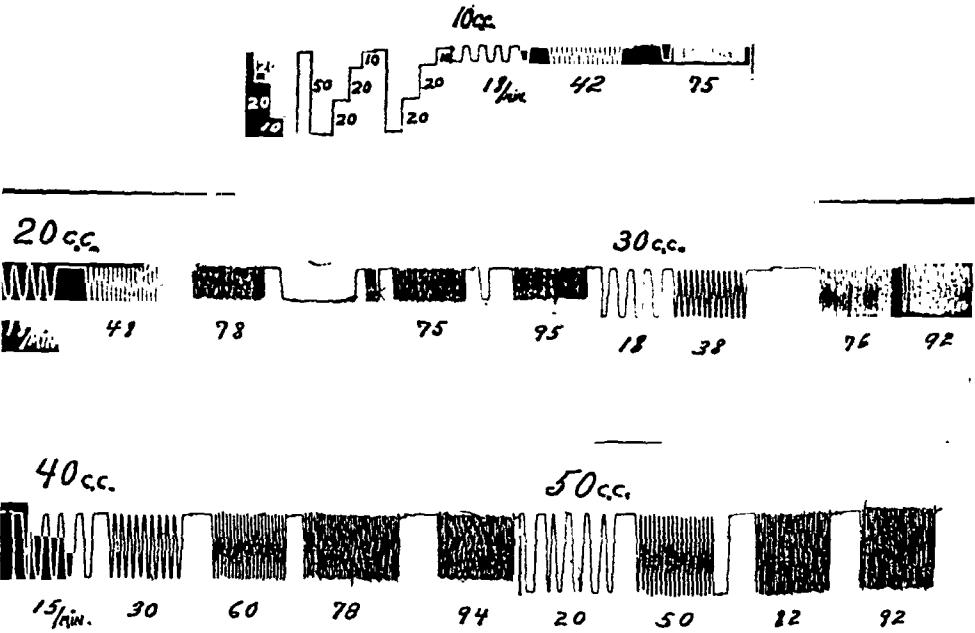


Fig. 4.—A record of the amplitude of excursion of the damped Krogh spirometer at the volumes and rates (per minute) indicated.

means of a hinged joint, to the counterbalance arm as shown in Fig. 3. This vane, immersed in oil as shown, effectively damped out all variations in the excursion of the spirometer at rates up to 100 per minute. The oil used is com-

mercial motor oil of a viscosity usually designated by S.A.E. 40. This oil critically damps the apparatus here described at room temperatures between 22° C. and 26° C.

A test of the pressures developed in the rebreathing tank showed that no significant change in pressure was introduced by the use of this damping vane.

Fig. 4 shows a record made by introducing volumes from 10 c.c. up to 50 c.c. at the rates indicated using the damped spirometer. As can be seen, there is no variation in depth with rate, and a plot of the excursion of the pointer on the spirometer against frequency at any one volume gives a straight line, parallel to the abscissa, up to frequencies of 100 or more.

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## COMPARISON OF THE KOLMER-WASSERMANN, KAHN, AND ROSENTHAL TESTS FOR SYPHILIS\*

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WITH SPECIAL REFERENCE TO THE USE OF THE LATTER IN THE PUBLIC  
HEALTH LABORATORY

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IN RECENT years there has been a growing tendency on the part of syphilologists and laboratorians not to rely on a single test for the serologic diagnosis of syphilis. Some laboratories have replaced the time-honored Wassermann test with some simpler method, such as the Kahn test, but it is probably safe to say that most laboratories today use some modification of the Wassermann, checking the results by means of one or more of the flocculation tests. Of these the Kahn is probably the best known. The Kline test has more recently come to the fore as a simple microscopic test; it has been recommended by Kolmer<sup>1</sup> as being superior to the Kahn as a check test. In some laboratories it is routine to use both macroflocculation and microflocculation tests to check the Wassermann results. The purpose of this study was to determine the applicability of the newer Rosenthal microscopic test for use as a check method.

In 1929, Rosenthal<sup>2</sup> described a microflocculation method which was technically much simpler than the Kline test in that neither serum nor antigen was diluted, either before or after mixing, and tabulated the results obtained by testing 1,066 serums by the new method and by the Wassermann test. He found that the new test gave more clear-cut reactions with the weakly positive and doubtful Wassermann serums. Since it required only a small quantity of serum, it could also be used where only finger blood was available. In 1932, Tulipan and Director<sup>3</sup> reported the results of the Wassermann and Rosenthal tests on 1,067 serums, mostly syphilitic. They found that the two tests agreed in 986 instances, disagreed in 81. No disagreements were noted in individuals not clinically syphilitic. Of the 81 disagreements on clinically syphilitic cases, the Wassermann was positive and the Rosenthal negative on 17; the Wassermann

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was negative and the Rosenthal positive on 64. The majority of these disagreements occurred in tertiary cases and patients who had received treatment. These authors felt that the Rosenthal test tended to become positive earlier in the disease and to remain positive longer under treatment than the Wassermann. They recommended it for emergency uses, such as testing donors for transfusions, also to rule out negative serums before testing by more complicated methods.

In 1933, Burdon and Duggan<sup>4</sup> reported tests on 1,235 serums by the Kahn and Kline methods; on 400 of these they also used the Hinton, Meinicke clarification, Sachs-Georgi and a modified Rosenthal test. On the basis of their results they did not recommend the Meinicke or Sachs-Georgi methods, while they felt that the Hinton test was inherently limited in its practicability. Concerning the Rosenthal test they stated: "Our brief experience with the Rosenthal test has led us to believe that it is a method of real value as a supplementary procedure and that it would be especially useful in those laboratories where the Kahn test is done routinely, since the Kahn alcoholic extract, the only necessary reagent except a 2 per cent cholesterol in acetone solution, would be on hand at all times. The method is simple, rapid and economical. The results show that it is practically as sensitive as the Kline test, and no false positives were recorded. The reactions, however, were by no means as clear-cut as those of the Kline method, and in tests with normal serums the emulsion may develop a diffuse granularity which one must learn by experience to disregard." The modification of technic described by these authors will be detailed later.

#### TECHNIC

As devised by Rosenthal, the test was conducted as follows: Capillary pipettes were drawn from glass tubing, being kept together to form matched pairs. With one pipette 4 drops of inactivated serum were blown into the depression in a hollow-ground slide; with the other one drop of the antigen was added to the serum. The two were then mixed with a glass rod and, after a short time, examined under the low power objective. This method was used without significant change by Tulipan and Director. For the present study the modification of Burdon and Duggan was used. By this method the test is carried out as follows:

Glassware: Slides, 3 in. by 2 in. with twelve paraffin rings are substituted for the hollow-ground slides. The paraffin rings are made according to the technic for the Kline test.<sup>5</sup> I have found that a piece of 22 or 24 gauge nichrome wire makes a more sturdy loop than the two turns of iron wire suggested by Kline. Slides with twelve depressions ground in would be a timesaver where large numbers of tests are to be run, but for most laboratories the cost would probably be prohibitive. One eastern supply house quoted such slides at approximately \$2.00, each.

Pipettes: Those used for the Wassermann or Kahn tests may be employed.

Antigen: No. I. Kahn standard alcoholic beef heart extract (not cholesterinized).

No. II. Two per cent solution of cholesterol in chemically pure acetone.

The stock solutions are kept at room temperature and mixed in equal parts for use. The mixed antigen keeps well for from one to two weeks. Rosenthal tinted the antigen with methylene blue, but this is not essential to ease in reading.

**Method of Test:** Five-hundredths cubic centimeters of undiluted inactivated serum is pipetted into the paraffin ring. More than twelve tests should not be set up at one time. A drop of the antigen mixture is then allowed to fall on the surface of each serum from a pipette delivering about 0.015 c.c. per drop. I have found that a Schick test (27 gauge) needle on a small syringe makes a very satisfactory substitute for the capillary pipette, delivering approximately the correct amount of antigen. The annoyance of having the tip of the pipette become broken, possibly in the midst of a series of tests, is also obviated. Magath<sup>6</sup> suggested sealing a needle into a glass tube for use as an antigen pipette for the Kline test; this expedient could also probably be successfully used in the Rosenthal method. The time of addition of antigen to the serums is noted, and each preparation stirred with a separate toothpick. They are then examined under the low power objective, focusing on the surface of the drop. A preliminary observation is made of the character of the granular field; the final reading is made within from five to eight minutes, never more than ten, from the time the antigen was added. A negative reaction appears as a granular suspension, evenly distributed; slight clumping is noted as doubtful; well-defined clumps as positive, and large clumps in a clear field as strongly positive. A clear distinction between 2, 3, and 4 plus cannot be made.

**Wassermann and Kahn Tests:** Kolmer's three-tube qualitative modification of the Wassermann test<sup>7</sup> was used as the basic test in the examination of all serums, and agreement with it was considered the criterion of efficiency. The Kahn test was performed in accordance with the directions given by Todd and Sanford,<sup>8</sup> using antigens obtained from the Difco Laboratories. Kahn tests on the first 707 serums in the study were made with the "standard" antigen, the remaining 483 were tested with the "sensitized" antigen.

**Interpretation of Results:** The majority of the blood specimens examined in this laboratory come from the Municipal Venereal Disease Clinic, and of these the largest percentage is from colored patients. Cases of anticomplementary Kolmers accompanied by agreement of the Kahn and Rosenthal tests were considered as absolute agreement. In case of discrepancy in the two latter tests, additional specimens were taken, if necessary after a provocative injection of neoarsphenamine, until either positive or negative Kolmer reactions were obtained. However, these cases of anticomplementary Wassermanns with disagreement in the two check tests are listed as separate classifications in Table I, in which the various combinations of results are tabulated. A doubtful Rosenthal reaction with a negative Kolmer and Kahn test was considered as negative, except in treated cases, but with a positive Kolmer and Kahn reaction it was considered positive. In treated cases a positive or doubtful Rosenthal, even with a negative Kolmer and Kahn test, was considered as indicating the necessity for further treatment.

## RESULTS OF TESTS

In this series of tests we have examined 1,185 specimens of blood and 5 of spinal fluid. Four of the latter were negative by all three tests, the fifth gave a 4 plus Kolmer, negative Kahn and doubtful Rosenthal. The results with the Rosenthal test as applied to spinal fluid are thus seen to be rather inconclusive, and further study along this line would be desirable; however, these five specimens are included in Table I, with the results on the 1,185 blood specimens. There were 836 specimens for diagnosis; these are designated "New" in the table. There were 290 for the result of treatment, and 64 were unclassified in this respect. Some of the patients classified as new were known to have received

TABLE I

| KOLMER            | KAHN | ROSENTHAL | NEW | TREATED | UNCL. | TOTAL |
|-------------------|------|-----------|-----|---------|-------|-------|
| Neg.              | Neg. | Neg.      | 536 | 146     | 45    | 727   |
| Pos.              | Pos. | Pos.      | 192 | 49      | 9     | 250   |
|                   |      |           | 728 | 195     | 54    | 977   |
| Pos.              | Neg. | Pos.      | 40  | 39      | 4     | 83    |
| Neg.              | Neg. | Pos.      | 10  | 23      | 3     | 36    |
| Pos.              | Neg. | Neg.      | 14  | 8       | 2     | 24    |
| Neg.              | Pos. | Pos.      | 4   | 11      |       | 15    |
| Neg.              | Pos. | Neg.      | 5   | 4       |       | 9     |
| Pos.              | Pos. | Neg.      | 3   | 1       |       | 4     |
| Ac.*              | Neg. | Pos.      | 2   | 3       |       | 5     |
| Ac.               | Pos. | Neg.      | 2   | 2       |       | 4     |
|                   |      |           | 80  | 91      | 9     | 180   |
| Neg.              | 0†   | Neg.      | 14  |         |       | 14    |
| Neg.              | 0    | 0         | 5   | 1       |       | 6     |
| 0                 | Neg. | Neg.      | 3   | 1       | 1     | 5     |
| 0                 | Neg. | Pos.      | 1   |         |       | 1     |
| Pos.              | Pos. | 0         | 2   |         |       | 2     |
| Neg.              | Neg. | 0         | 2   |         |       | 2     |
| Pos.              | 0    | 0         | 1   |         |       | 1     |
| Ac.               | 0    | Pos.      |     | 2       |       | 2     |
|                   |      |           | 28  | 4       | 1     | 33    |
| Total (all tests) |      |           | 836 | 290     | 64    | 1,190 |

\*Ac.—Anticomplementary.

†0—Test not made.

TABLE II

|                   | KOLMER |        | KAHN  |        | ROSENTHAL |        |
|-------------------|--------|--------|-------|--------|-----------|--------|
|                   | NO.    | PCT.   | NO.   | PCT.   | NO.       | PCT.   |
| Positive          | 364    | 30.59  | 284   | 23.86  | 389       | 32.69  |
| Negative          | 809    | 67.99  | 883   | 74.20  | 790       | 66.39  |
| Anticomplementary | 11     | 0.92   |       |        |           |        |
| Not done          | 6      | 0.50   | 23    | 1.94   | 11        | 0.92   |
| Total             | 1,190  | 100.00 | 1,190 | 100.00 | 1,190     | 100.00 |

treatment from one to twenty years previously, but their blood tests were not made as a check on such treatment, but rather for diagnostic purposes.

The first division of Table I records the tests which may be considered as absolute agreement; the second division those in which disagreements occurred



among the three methods; and the third division those specimens on which one or two of the tests were not done, due usually to an insufficient amount of serum, breakage of tubes, etc.

Consideration of Tables I and II shows that the Rosenthal test is apparently more sensitive than either of the other two, especially in the cases of patients who have received treatment. It will also be noted that the greatest number of discrepancies falls in the class of treated cases. These findings are in accord with those obtained by Tulipan and Director. Contrary to these authors and also to Burdon and Duggan, Table I shows a number of examples of what must have been false positives although several of the serums may have been from early cases of syphilis or from patients who had previously received treatment.

Of the 180 disagreements noted in the second division of Table I, 147 showed negative Kahn tests with positives by either one or both of the other methods. Of this group, 94 were included in the 707 specimens examined with the standard antigen, while 53 were part of the 483 tests made with the sensitized antigen. This gives percentages of 13.2 and 10.9, respectively. This reduced sensitivity of the standard antigen was also remarked by Burdon and Duggan.

From the standpoint of practicability, it is desirable to know what percentage of agreement may be expected from the tests under consideration, or from various combinations of them. These figures are tabulated in Tables II and III.

TABLE III

| IN AGREEMENT               | NUMBER | PERCENTAGE OF TOTAL |
|----------------------------|--------|---------------------|
| Kolmer, Kahn and Rosenthal | 977    | 82.1                |
| Kolmer and Rosenthal       | 1,033  | 91.0                |
| Kolmer and Kahn            | 1,021  | 85.7                |
| Kahn and Rosenthal         | 1,021  | 85.7                |

## SUMMARY AND CONCLUSIONS

Results are presented covering the examinations on 1,185 specimens of blood serum and 5 spinal fluids by the Kolmer-Wassermann, Kahn, and Rosenthal tests. Comparisons are tabulated, giving the percentages of agreement between the various combinations of tests. On the basis of these results, we may conclude that the Rosenthal test is an accurate, simple, and economical method, agreeing well with the Wassermann and Kahn tests. On these grounds it can be recommended as a check test, or as a preliminary test, to be checked later by other methods. It is especially applicable to the public health laboratory, where the short time requirement (twelve tests may be set up and read in ten minutes) will commend it to most laboratorians. The fact that it may be performed with finger blood and in such a short time makes it a valuable procedure for use in the hospital laboratory as an emergency method, such as for testing donors for transfusions. In this connection it is to be remembered that the antigen may be kept on hand and a single test set up without any bother of making dilutions or waste from preparing a quantity of reagent. It may also

be found useful in the routine suggested by Tulipan and Director, that of testing first by some simple method to rule out the frank negatives, then testing positive and doubtful serums by other methods to confirm or disprove the preliminary findings.

As with any study of this type, the results obtained give rise to other interesting problems which must be passed up in this laboratory. The question of whether the positive Rosenthals with negative Kolmer and Kahn reactions in new patients are really false positives or whether they are incipient syphilis or beginning cerebrospinal syphilis, could be solved by the aid of a well-organized social service follow-up work. It would also be interesting to tabulate such a series as this one on the basis of the part of the body involved, that is, visceral, cerebrospinal, bone, etc. This could best be done in a large hospital center.

The author wishes to express his appreciation to the following men for their criticism of the manuscript during its preparation: Dr. G. D. Johnson, Dr. H. H. Wescott, Dr. K. D. Graves, Dr. W. F. Manley, Dr. W. C. Stephenson, Jr., and Dr. K. T. Redfield.

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## AN AUTOMATIC-FILLING INK RECORDING SYSTEM AND PERMANENT TYPE WATER MANOMETER\*

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**A**N INK recording system on apparatus such as the water or mercury manometer, signal magnet, etc., has distinct advantages over the ordinary smoked paper recording method. The apparatus indicated in Fig. 1 is sturdy, durable, simple, and of light weight. The ink recorder and the permanent type water manometer (Fig. 2) have been continuously used with satisfaction in balloon studies of gastrointestinal motility for the past two years. A manometer having the dimensions indicated is satisfactory for many purposes, and manom-

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\*From the Department of Physiology, Western Reserve University Medical School.  
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eters of this type, which are exact duplicates or definite variations, can be made to subserve special purposes. Difficulties related to air leaks are largely eliminated, and the necessity of making replacements is decreased by the avoidance of most of the rubber tubing employed on ordinary manometers.

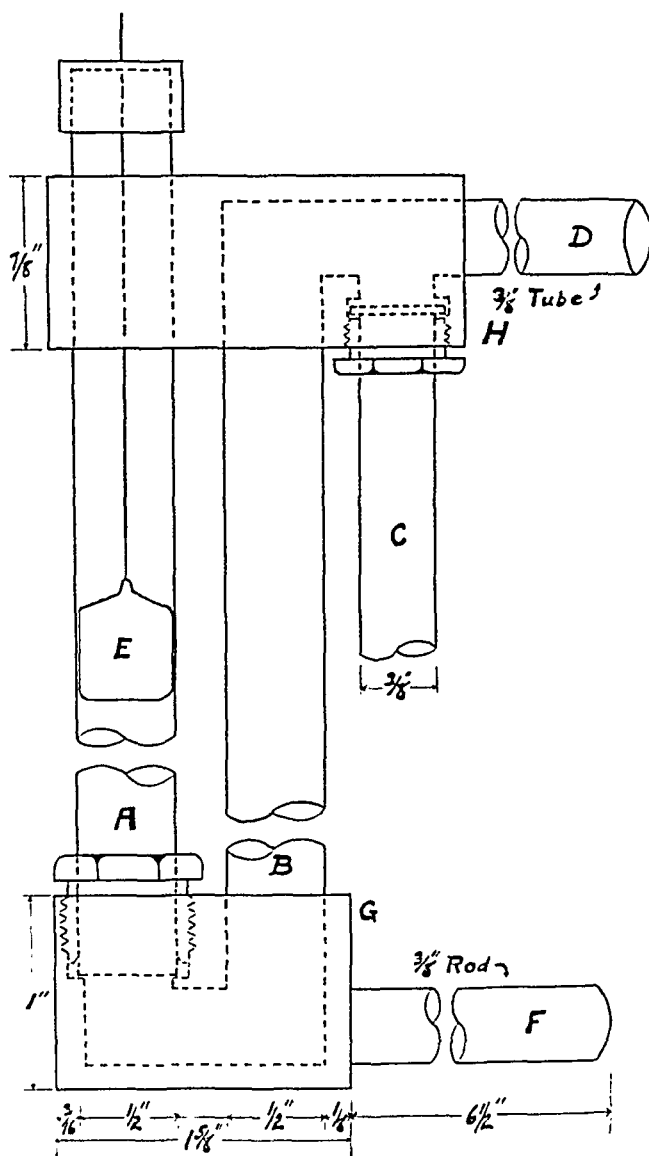


Fig. 2.—Permanent type water manometer. Glass tube *A* and brass tube *B* set into brass blocks, *G* and *H*; *C*, tube of soft copper or lead (reaches to balloon tube inserted in animal); *D*, brass tube sweated into *H* and fitted at outer end with short length of rubber tube and clamp (employed in adjusting pressure within system); *E*, thin-walled glass float sealed to float rod of flexible wire and light weight capillary tubing; *F*, brass rod sweated into *G* (used with *D* to support manometer).

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

UNDULANT FEVER, "Brucellin," a Possible Specific for, Huddleson, I. F., and Johnson, H. W. *Am. J. Trop. Med.* 13: 485, 1933.

The authors comment upon the various pitfalls surrounding the accurate diagnosis of *Brucella* infection, emphasizing the uncertainty of blood cultures, especially in infections of the suis or abortus varieties, and the difficulties of accurate interpretation of agglutination titers.

They have developed what they consider a satisfactory procedure for the detection of active *Brucella* infection utilizing both an allergic skin reaction and a determination of the opsonophagocytic power of the patient's blood.

These procedures are carried out as follows:

*Brucella Allergic Skin Test.*—The test solution is a soluble nucleoprotein fraction made from the three species of *Brucella* in 1:1,000 dilution in slightly alkaline physiologic saline.

The test is made by injecting about 0.1 c.c. of the fluid intracutaneously in the upper anterior surface of the forearm using a 26 gauge needle. The size of the local reaction, which is characterized by a circumscribed erythema and edema, may vary from 1 to 2 inches in diameter and appears within from four to forty-eight hours after injection. It may persist for from forty-eight to ninety-six hours. There is invariably no necrosis or sloughing of the tissue at the point of the local reaction. In the infected, the local reaction may be accompanied by a more marked manifestation of symptoms. Those that are hypersensitive will show a severe systemic reaction along with the local reaction. Those that have not been sensitized to *Brucella* and who are probably susceptible to infection show no local or systemic reaction. One often sees in certain individuals an erythema, about  $\frac{1}{2}$  to 1 inch in diameter with no edema around the point of the injection. It has the appearance of a nonspecific reaction.

*Determination of Opsonophagocytic Power of Blood.*—(See abstract published in June issue, page 1024: Huddleson, Johnson, and Hamann, "A Study of the Opsonocytophagic Power of the Blood and Allergic Skin Reaction in *Brucella* Infection.") This is a modification of the Leishman-Veitch technic and consists in mixing equal quantities of a citrated (0.8 per cent sodium citrate) fresh blood and a heavy bacterial suspension of living organisms in a small Wassermann tube, incubating at 37° C. for thirty minutes, and subsequently making spreads and staining with Hastings' stain. The addition of a definite amount of sodium citrate prevents clotting of the blood and inhibits the action of *Brucella* opsonins which are present in the serum of many normal individuals.

This paper is particularly concerned with a report of the therapeutic results obtained with "Brucellin," a product developed by the authors which may possibly prove a specific for the treatment of undulant fever.

This agent is prepared by growing each of the three species of *Brucella* in separate flasks of beef liver infusion broth, adjusted to a  $P_H$  of 6.6, for sixty days at 37° C. The inoculated flasks are shaken vigorously at weekly intervals. At the end of the stated growing period, each flask is examined bacteriologically and, if found contaminated, is discarded. The broth is clarified by centrifugation at a speed of 3,000 r.p.m. for four hours. The clear liquid is decanted and adjusted to a  $P_H$  of 7 with N/1 HCl. The product from each of the three species is now pooled and filtered twice through sterile Berkefeld N filters. The filtered liquid is transferred to sterile flasks or tubes and incubated for five days at 37° C. to determine sterility. It is standardized and transferred to sterile 1 c.c. vials or stored in larger containers until ready for use. The duration of its potency has not been fully determined.

When a certain diagnosis of undulant fever has been made, an intradermal injection of about 0.05 to 0.1 c.c. of "Brucellin" should first be given to determine the sensitiveness of the patient to this material. If no marked systemic reaction is elicited within forty-eight hours after the intradermal injection, one may give 1 c.c. intramuscularly. This dose should be repeated at intervals of three days until four injections are given. The intramuscular injections usually cause a local reaction and the temperature of the patient to rise to 104 or 105° F. About one to three hours after the temperature reaches its highest point, it begins to fall by steps or may rapidly drop to near normal. There is as a rule an increase in the severity of the symptoms, such as headache, muscular pains, chills, and sweating. The increase in the severity of the symptoms usually does not persist for more than twenty-four hours. The temperatures of patients treated within the fourth week after the onset tend to return to normal or subnormal after the second injection.

If the systemic reaction from the intradermal injection is quite severe, it is advisable to start the intramuscular injections with 0.1 or 0.2 c.c. If there is no severe systemic reaction following the intramuscular injection of the smaller amount, the succeeding dose at the end of the three days may be doubled. Each dose thereafter may be doubled, providing the systemic reaction is not severe, until 1 c.c. is reached. Three successive 1 c.c. injections may then be given. Certain individuals show very little systemic reaction to 1 c.c. amounts of "Brucellin." Such patients do not respond to treatment until after the dose is increased to 3 or 5 c.c. amounts.

In case children are treated with "Brucellin," the beginning dose should be 0.2 c.c. If the reaction which follows is not severe, each succeeding dose at three-day intervals may be doubled until 1 c.c. is reached. Three successive 1 c.c. injections may then be given.

The ambulatory or chronic type of case of long standing does not respond as readily to four 1 c.c. injections of "Brucellin" as those of three months' duration and less. It may be necessary to gradually increase the dose to 5 c.c. by 1 c.c. steps in order to effect recovery in cases of long standing. Several cases, however, of from eight to twelve months' duration have responded very readily to four injections of "Brucellin." The response to treatment made by infected infants and children, including those in which the disease has assumed the meningeal form, is very striking.

One should not try to avoid systemic reactions from injections of this material. It is necessary to produce two or three systemic reactions in order to bring about recovery.

If it is possible, one should determine the opsonocytaphagic activity of whole citrated blood for *Brucella* after the fourth injection as it has been observed that the phagocytic activity of the blood for *Brucella* is one of the surest criteria of complete recovery from infection. In the absence of such a test one must be guided in determining recovery by the disappearance of the clinical manifestations of the disease, by the appearance of a subnormal temperature, change in mental attitude of the patient and marked increase in the appetite.

Because of the danger of severe systemic reactions as a result of sensitization, it is essential that "Brucellin" be used only for active cases and never injected into a recovered patient. The necessity for accurate distinction between a recovered patient—recognized by the presence of agglutinins, for example—and an active infection is obvious and cannot be overemphasized.

"Brucellin" has been used in the treatment of 80 cases with excellent results.

#### SWIMMING POOLS, Some Factors Involved in the Use of Chloramines for the Disinfection of, Fletcher, A. H., and Link, E. C. *Am. J. Pub. Health* 23: 1255, 1933.

The decided advantage in the use of chloramines for disinfection of swimming pools having a slow rate of recirculation, poor spacing of inlets, hand application of chlorine, or those operated as fill-and-draw pools, and especially when the pool is out of doors, has prompted its use for another season.

The use of chloramines instead of chlorine alone requires certain extra tests and control to guard against its limitations. Some of these are: (a) the requirement of higher

residuals, that is from 0.7 to 1 p.p.m.; (b) the control of the ratio of ammonia to chlorine and of the free ammonia content in the water; (c) the control of the  $P_H$  of the water routinely; and (d) the collection of all samples in sodium thiosulphate-treated bottles.

The duplicate series of tests on ordinarily collected samples and dechlorinated collected samples showed a lag in the killing power of chloramines over the chlorine on *B. coli*.

A moderate excess of sodium thiosulphate when used as an antichlor was found to have no disinfecting effect.

Copper sulphate, if added every second night after closing at a dosage rate of 5 lb. to each 100,000 gal. of water, definitely and completely controlled algae or slime growth at any pool where it occurred in Memphis.

**STREPTOCOCCI, Hemolytic Properties of the Mastitis, Hansen, P. A., Hucker, G. J., and Snyder, M. A. Am. J. Pub. Health 23: 1262, 1933.**

Of 91 authentic strains of *S. mastitidis* being carried in various laboratories as typical streptococci associated with mastitis 50 were of the alpha and 41 of the alpha prime hemolytic types.

The streptococcus commonly associated with chronic or subclinical mastitis always produces the alpha or alpha prime type of hemolysis on blood agar, bordering on a slight beta hemolysis. Strong beta hemolytic strains were not found among the cultures. Two strains of streptococci from mastitis were beta hemolytic, but they were found by cultural studies to be different from the type generally found associated with mastitis.

A careful study of the authentic mastitis streptococci isolated from chronic cases reported to be beta hemolytic were found to be of the alpha prime type. This distinction is apparent when deep colonies are examined by a stereoscopic binocular microscope.

Blood from sheep, ox, and horse was used with similar results. The type of blood did not appear to affect the type of hemolysis produced on blood plates.

**PREGNANCY, The Sex Determination Test of Dorn and Sugarman, Curphey, T. J., and Romer, A. S. J. A. M. A. 101: 1680, 1933.**

From their experimental study the authors conclude: It is evident that there is no constant agreement whereby the changes excited in the testicle of the rabbit can be used to predict the sex of the unborn child.

Using a pure bred strain of New Zealand white rabbits, they were unable to confirm the observations of Dorn and Sugarman as to the prediction of sex in the unborn child.

The age of the experimental animal and the anatomic location of the testicle at the time of intravenous injection of urine of pregnancy apparently plays no part in the matter of testicular stimulation.

There is present in the urine of pregnant women a so-called spermatogenic factor unassociated with the sex of the unborn child.

The need exists for a further investigation as to a possible relationship between this spermatogenic factor and toxemic states of pregnancy.

**TUBERCULOSIS, Effect of Vaccination With BCG on Children From Tuberculous Families, Park, W. H., Kereszturi, C., and Mishulow, L. J. A. M. A. 101: 1619, 1933.**

From the results of these investigations it appears that B.C.G. is so attenuated that even under the most favorable conditions of artificial cultivation it is difficult to increase its virulence to any degree. During the past five years, the virulence of the B.C.G. vaccine was tested on 165 rabbits and 194 guinea pigs but no evidence was found of any increase in virulence. Twenty cultures of acid-fast bacilli that were recovered from the cold abscesses of seventeen children after from one to six months' stay in the body showed no increase of virulence, but remained like the B.C.G. in colony morphology and were nonvirulent for rabbits and guinea pigs.

One culture was recovered from the mesentery of an orally vaccinated child six weeks after vaccination. This culture showed no increased virulence and culturally was typically like B. C. G.

The pathologic material that was obtained from children who were vaccinated with B. C. G. and died of other infections showed no evidence that B. C. G. tended to increase in virulence during its residence in the human body.

On the basis of their limited experience and on the basis of publications of others, the authors do not think that slight primary tuberculous infection acquired by natural infection or produced by vaccination diminishes resistance against future superinfections by tuberculosis.

Children of tuberculous families vaccinated orally with B. C. G. show lower mortality from tuberculosis than corresponding controls.

None of the parenterally vaccinated children died of tuberculosis, whereas the children who were not vaccinated and were similarly exposed showed about 3 per cent mortality from tuberculosis. There was no appreciable difference in the mortality of the controls who had negative roentgen findings in the chest when they were first seen, whether they had negative or positive initial tuberculin tests.

Tuberculin tests performed on 148 nonvaccinated children followed up since birth and on 205 children orally vaccinated with B. C. G. before their tenth day showed, during the first two years of life, from 20 to 40 per cent more positive Mantoux tests among the latter cases. The allergy produced by B. C. G. apparently does not usually last for more than two or three years.

Comparatively few of the control children developed positive reactions to tuberculin, even when they were more or less exposed to open tuberculosis, i.e., 20 per cent in the first year and 50 per cent up to the fourth year.

If repeated tuberculin tests are performed through the years, a small percentage of the slightly or moderately positive reactions become negative.

**NEPHRITIS, Types of and Their Management, Christian, H. A. J. A. M. A. 102: 169, 1934.**

The present study of lipoid nephrosis and the so-called "nephrotic syndrome" suggests that lipoid nephrosis, in adults at least, is a form of Bright's disease.

The frequent association of lipoid nephrosis with definite glomerular nephritis, the fact that cases of clinically pure lipoid nephrosis may terminate in uremia from the end-stages of glomerular nephritis, and the fact that not a single case typical of lipoid nephrosis in an adult has come to postmortem examination at the Mayo Clinic suggest that at least most cases of lipoid nephrosis represent a stage in, or an unusual type of, glomerular nephritis. If other cases occur, they are extremely rare.

The relatively high percentage of patients who have been cured of lipoid nephrosis, or who have shown marked and prolonged improvement, and the relatively slight evidence of glomerular nephritis in most instances at postmortem examination, even among patients who have given clinical evidence of glomerular nephritis, justify the grouping of these patients clinically in a separate group from that of ordinary glomerular nephritis. As long as the term lipoid nephrosis has been so uniformly accepted there seems to be no good reason for changing it.

**SERUM PROTEINS, In Diseases not Primarily Affecting the Cardiovascular System or Kidneys, Peters, J. P., and Eisenman, A. J. Am. J. M. Sc. 186: 808, 1933.**

From an analysis of 109 total protein determinations from 52 individuals and of 50 protein fractions from 34 individuals, the following limits of normal variation have been defined: for total protein, from 6 to 8 per cent; albumin, from 4 to 5.5 per cent; globulin, from 1.4 to 3 per cent. Globulin in excess of 2.5 per cent was found only in the winter months and may represent effects of respiratory infections which are so prevalent in this climate at that season.

Total proteins have been determined 542 times in 374 patients suffering from various diseases which do not affect directly the cardiovascular or renal systems, and in 215 of these subjects 332 determinations of protein fraction have been made. The data have been analyzed from various points of view and the following conclusions have been drawn.

The relation between edema and the estimated protein osmotic pressure is in keeping with the theory of Starling.

Reductions of albumin can be connected almost invariably with malnutrition.

Increases of globulin are found with infections, especially suppurative processes and ulcerative tuberculosis or syphilis; in connection with certain tumors, particularly myelomata, and in cirrhosis of the liver.

The study has thrown no light on the sites of production of the two protein factors.

**TRICHINIASIS, The Use of an Intradermal Test in the Diagnosis of, McCoy, O. R., Miller, J. J., Jr., and Friedlander, R. D. J. Immunol. 24: 1, 1933.**

Of 36 persons ill with trichiniasis (i.e., two to six weeks after infection) 25 (70 per cent) gave positive immediate skin reactions following intradermal injection with 0.1 c.c. of a 1:10,000 dilution (in terms of dry weight of powder) of a saline extract of *Trichinella spiralis* larvae. An additional 8 reacted to a 1:500 dilution of the antigen making a total of 33, or 92 per cent positive.

Of 39 persons tested from three to twenty-two months after infection, 19 (49 per cent) reacted to the 1:10,000 dilution and an additional 12 were positive to a 1:500 dilution, a total of 31 or 80 per cent positive.

Of 13 persons ill from three and one-half to seven and one-half years prior to the test, 3 (23 per cent) were positive to the 1:10,000 dilution, and 5 more reacted to the 1:500 dilution, a total of 8 or 62 per cent positive.

One hundred and four control persons from Rochester, N. Y., showed an incidence of 9 per cent positive with the 1:10,000 dilution and a total of 18 per cent positive with both the 1:10,000 and 1:500 dilutions. Forty-seven control persons from San Francisco, Calif., showed an incidence of only 4 per cent positive to the 1:10,000 antigen and 6.5 per cent positive with both the 1:10,000 and 1:500 dilutions.

Ninety-two persons from southern Louisiana infected with *Trichuris trichiura* showed an incidence of 18 per cent positive with the 1:10,000 dilution and a total of 62 per cent with both the 1:10,000 and 1:500 dilutions.

From the data given in the present paper it appears fairly well established that about 90 per cent of persons ill with trichiniasis will give a positive skin test to the trichinella antigen provided the disease is sufficiently established. This period is apparently between two and three weeks after infection although in some cases a positive skin test may not appear until the fourth week.

The first consideration which complicates the interpretation of a positive trichinella skin test is the possibility that a previous light infection which has nothing to do with the present illness is responsible for the sensitization. This possibility is somewhat reduced by the fact that there seems to be a tendency to lose sensitivity over a period of years following infection.

Another consideration which must be thought of in the interpretation of a positive trichinella skin test is the possibility that the reaction is a Group I due to infection with *Trichuris trichiura*. The data show that most of these group reactions occurred with the 1:500 dilution of the antigen, but that a few probably occurred also with the 1:10,000 dilution. From the series of control tests performed, the authors are of the opinion that worm infections, other than trichuris, allergic disturbances, or other ordinary diseases are not an appreciable factor in causing group reactions to the 1:10,000 dilution of the trichinella antigen. It is possible, however, that in isolated instances group reactions may occur. In such cases it would probably be advisable to perform control tests with other proteins.

From a practical standpoint the trichinella skin test cannot be considered a certain diagnostic test. It appears that a negative test will probably prove more useful in ruling



out the diagnosis of trichiniasis than a positive one in establishing it. A positive test, however, definitely strengthens the evidence for the diagnosis and may justify further search for the parasite. Positive tests obtained with antigen dilutions of 1:10,000 or higher are of more definite diagnostic value than those obtained with lower dilutions. A year's experience in using the skin test in hospitals where trichiniasis not infrequently occurs leads the present authors to the conclusion that it may be a valuable aid in the diagnosis of the disease.

**TRICHINOSIS, A New Jersey Outbreak of, With Report of a Case Complicated by Femoral Thrombosis, Kilduffe, R. A., Barbash, S., and Merendino, A. G.** *Am. J. M. Sc.* 186: 794, 1933.

A case of trichinosis complicated by femoral thrombosis is reported together with the particulars of an outbreak arising from the ingestion of homemade sausage and involving 43 persons.

**TRICHINOSIS, Bachman Intradermal Test in Human, Kilduffe, R. A.** *Am. J. M. Sc.* 186: 802, 1933.

The results of the application of Bachman's skin test applied to 33 individuals exposed to trichinosis are reported and analyzed, from this analysis the author concluded that in the study of human trichinosis:

1. The demonstration of eosinophilia is not only technically simpler than the demonstration of the skin test, but always feasible, whereas the skin test, requiring an antigen difficult to prepare, is feasible only when the antigen is available.

2. In point of delicacy and constancy of appearance eosinophilia serves as a reliable index of trichinosis in the human being.

3. The Bachman skin test in the study of human trichinosis presents no practical advantages over the demonstration of eosinophilia.

**NEUTROPENIA, Benign and Malignant, Beck, R. C.** *Arch. Int. Med.* 52: 239, 1933.

Because of its comprehensive survey of the subject this paper should be read in the original, as its character precludes a satisfactory abstract. The summary following indicates the completeness of this review, which is followed by a report of 4 cases.

The subject of benign and malignant neutropenia has been covered, and this information has been compiled in more or less textbook fashion.

Many names have been proposed for this disease entity, benign and malignant neutropenia seeming to be the most appropriate.

The two groups, benign neutropenia and malignant neutropenia, have been further subdivided into primary and secondary types, depending on the etiology. A classification is based on this method of distinction and on the clinical course, which tends to give a mental picture of the types that make up this disease.

A review of the physiology of granulopoiesis is given, since the accuracy in interpreting the blood picture in primary benign and malignant neutropenia is dependent on an understanding of the underlying mechanism of granulopoiesis.

A review of the theories of pathogenesis of benign and malignant neutropenia is covered, and a theory is formulated based on the underlying pathology and physiology of granulopoiesis. It is suggested that in each case of neutropenia it would be advisable to determine the etiology and underlying changes in the bone marrow. Biopsies on specimens of bone marrow in all cases before treatment is given would prove or disprove the theory of an endogenous disturbance of chemotactic and maturation factors for granulocytes as being the cause of this disease.

The pathology of the characteristic lesions has been covered, and complete laboratory data given.

Symptoms, diagnosis, differential diagnosis, and prognosis have been discussed.

A section is devoted to a theoretical consideration of the treatment for this disease, as well as a review of all forms of treatment to be found in the literature. The rationale of these treatments, when possible, has been discussed. The theory is advanced that a specific treatment for malignant neutropenia involves an investigation of the chemical maturation factors which determine the granulocytes.

**SILICOSIS, The Pathology of So-Called Acute, Gardner, L. U.** *Am. J. Pub. Health* 23: 1240, 1933.

A histologic study of 15 cases of so-called "acute" silicosis has been reported. This was based upon examination of the lung tissues of 9 tunnel workers exposed for from nine to thirteen months, 3 sand blasters, for twelve to seventeen months, and 3 sand pulverizers on whom satisfactory histories of exposure were not obtained. Chemical analysis demonstrated that their lungs contained as much silica as those of South African gold miners employed for long periods. Their alleged occupational exposures did not exceed seventeen months, a contention which is supported by the early age of the group as a whole. These men died of infection from nine to twenty-one months after ceasing work. In 11 of them the infection was considered to be definitely tuberculous, and in 2 others it was probably so. The other 2 had unresolved pneumonias. All but 1 of them, a tunnel man for thirteen months, presented microscopic lesions characteristic of silicosis, but in no case was the disease sufficiently far advanced to be diagnosed on gross examination of the fixed tissues. The character and distribution of the changes did not resemble that usually seen in silicosis among miners and others who have died after prolonged exposures. This silicosis was characterized: (1) by masses of small nodules embedded in broad sheets of fibrous tissue surrounding the pulmonary lymphatics instead of isolated conglomerate nodules along the trunks and in the pulmonary parenchyma; (2) by a generalized fibrous thickening of the alveolar walls, which is generally considered to be a late manifestation; and (3) by the absence or only slight involvement of the mediastinal lymph nodes which are usually replaced by fibrosis before extensive changes appear in the lung. These differences were interpreted as due to the inhalation of such excessive amounts of fine dust that little of it could be eliminated by the lymphatics. In most cases the tuberculous complication was so acute that it probably developed after the reaction to the dust was well established, and thus failed to influence the picture greatly.

The acuteness of the infection was explained by the fact that the majority of the men were young negroes with presumably little immunity to tuberculosis. Although there is histologic evidence of silicosis, atypical in character, it seems doubtful whether there is justification for describing the process as "acute." At least this should only be done after serial roentgenograms together with postmortem examinations have demonstrated the outcome of the allegedly heavy exposures to silica. The difficulty in properly evaluating the element of infection in the tissues suggests the need for caution in the interpretation of roentgenograms.

**SILICOSIS, Silica Content of Lungs of a Group of Tunnel Workers, Smith, C. S., and Wikoff, H.** *Am. J. Pub. Health* 23: 1250, 1933.

The lungs of 9 workmen engaged in the same tunnel project have been analyzed.

There is a striking correlation between the per cent  $\text{SiO}_2$  in the dried lung substance and the severity of silicosis as determined histologically.

The per cent  $\text{SiO}_2$  in the dried lung substance is a better criterion of the intensity of silicosis than is the per cent of silica in the total ash.

**BLOOD, Is Halometry Reliable? Chaudhuri, H. P.** *Indian J. M. Research* 21: 315, 1933.

From an extensive statistical and comparative study, it is concluded that the halometric determination of the size of erythrocytes furnishes reliable data.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### An Outline of Immunity\*

AS THE author pertinently remarks in his Preface, there is probably no subject in a less satisfactory position than that labeled "Immunity." "A hybrid creature, not quite sure of its true affiliations; wandering uneasily between the Departments of Pathology, Bacteriology, and Hygiene; at times attempting to set up a house all by itself; a vigorous infant among the sciences, just rising fifty years, like most young and vigorous things, it is a little uncertain in its movements, a little confused in its mind, and at times more than a little incoherent in its speech."

Few who are familiar with the many, and at times not a little startling developments in the field of immunity within comparatively recent times, will deny the aptness of Professor Topley's characterization of his subject. That he has been able to present as clear and as comprehensive a survey of so complicated a subject as he has given in the present volume is a matter upon which the medical profession may well congratulate him and itself.

This is a book which should prove of inestimable value not only to the student and laboratory worker, but to the physician at large who, obliged almost daily to utilize the phenomena of immunity in the treatment and prophylaxis of disease, is obliged also to do so often without clear apprehension of the principles involved and their practical application.

In the twenty-one chapters of this volume the present concepts of immunity are reviewed, summarized, and correlated in a comprehensive and authoritative way. The book, far from being abstruse and concerned with hypothesis, is eminently practical in its outlook and consistently concerned with the practical aspects of immunity as related to the prophylaxis and treatment of disease.

An excellent feature is the succinct yet clear summary at the end of each chapter which, together with the excellent index, renders the contents of the volume readily accessible.

This book may be highly recommended as serving a useful purpose and as presenting a highly practical survey of an important and complicated subject.

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### Coccidia and Coccidiosis†

COCCIDIA, in their relation to disease in animals and man, have attracted attention in many and diverse fields as witnessed by the numerous papers concerning coccidiosis which have appeared in journals of various types.

This field, however, is a highly specialized one, the literature of which is so widely scattered as to be almost inaccessible to even the specialist with good library facilities.

This monograph, therefore, serves a most useful purpose in bringing together under one cover descriptions and illustrations of the different coccidia found in various hosts and presents, in addition, the results of investigations in the study of "host-parasite-relationships."

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\*An Outline of Immunity. By W. W. C. Topley, Professor of Bacteriology and Immunology, University of London. Cloth, pp. 415, 37 figures, 63 tables. William Wood & Co., Baltimore, Md.

†Coccidia and Coccidiosis of Domesticated, Game, and Laboratory Animals, and of Man. By Elery R. Becker, D.Sc., Associate Professor of Protozoology, Iowa State College. Cloth, pp. 147, 25 figures. Collegiate Press, Ames, Iowa.

Throughout the book much attention has been paid to discussions of those phases of coccidiosis which have been the subject of misconception in the past, such as host-specificity, the nature of species, the relationship to disease, immunity, methods of reducing infection, and the tolerance of the oocysts.

There are numerous illustrations and an appendix on technique together with an extensive bibliographic index and a general index render the contents of the book readily accessible.

To the biologist, the pathologist, the practitioner, and the breeder, this book should be of interest and value as correlating in a systematic manner the present information available concerning a subject about which there has been much confusion in the past.

### Recent Advances in Vaccine and Serum Therapy\*

THE purpose of this book is to survey the present state of knowledge regarding the employment of vaccines and sera in the prevention and treatment of disease.

Few will deny that there has been and still is more or less clinical confusion in this field and the present volume is, therefore, not only timely but fills, as well, a place long empty in the physician's reference library.

The book is divided into two parts. Part I, written by Dr. Petrie, is concerned with the prevention and treatment of disease by means of sera and with the specific prophylaxis and treatment of virus diseases in man and animals.

Part II, written by Professor Fleming, surveys the prevention and treatment of disease by means of vaccines.

An extraordinary amount of work has been done in both these fields, and the physician, laboratory worker, and student here finds it admirably surveyed and correlated.

The authors have not been satisfied merely to list or abstract the numerous investigations which have influenced present concepts in these fields, but present them in the light of their own experience, as a coherent and authoritative story, well conceived and well written.

The list of references presented at the end of each chapter evidences a careful and painstaking review of the literature and the extensive subject index as well as a complete and separate author index render the contents of the book readily accessible.

This volume should be on the reference shelf of every worker interested in this field and may be classed as indispensable for the physician who desires to utilize either sera or vaccines in an intelligent manner.

It may confidently be accepted as a valuable as well as highly practical reference work in this important field and recommended without reserve to the practicing physician.

### Manual of Determinative Bacteriology†

THE *Manual of Determinative Bacteriology* sponsored by the Society of American Bacteriologists and edited by Dr. Bergey has long been indispensable to clinical and bacteriologic laboratories and requires neither introduction nor labored comment.

This new fourth edition is enlarged by nearly 100 pages by amplification in the description of species and by the addition of about 50 species and one new genus.

Despite the thorough revision and the increased size of the book the price is unchanged. Were it twice as much the book would still be invaluable.

\*Recent Advances in Vaccine and Serum Therapy. By Alexander Fleming, Professor of Bacteriology, University of London, and G. F. Petrie, Bacteriologist-in-Charge, Serum Department, Pasteur Institute, Elstree. Cloth, pp. 463. 5 illustrations. P. Blakiston's Son and Co., Philadelphia, Pa.

†Manual of Determinative Bacteriology. By David H. Bergey. Cloth, pp. 664. Fourth edition. Williams & Wilkins Co., Baltimore, Md.

## Practical Methods in Biochemistry\*

WHILE intended primarily for medical students this manual should also prove useful to the physician, not only as a technical manual, but also as a work of reference concerning the principles and methods of biochemistry which now occupy so prominent a place in the practice of medicine.

The style is clear, simple, and easily understandable and the volume may be recommended as a comprehensive and reliable guide.

## A Textbook of Bacteriology†

FEW textbooks of bacteriology have attained a more lasting or more deserved popularity than that of Hiss and Zinsser, the new seventh edition of which, prepared by Zinsser and Bayne-Jones and dedicated to the memory of Dr. Hiss, now makes its appearance.

So familiar is this excellent text as a comprehensive and authoritative presentation of this subject that little need be said by way of introduction.

While extensively revised and in many ways rewritten, the original purpose of the book has been maintained, to present not only a treatise on the fundamental laws and technique of bacteriology, as illustrated by their application to the study of pathogenic bacteria, but also to serve as a manual of infectious diseases.

The scope of the work is indicated by the titles of the nine main sections into which it is divided: Part I, The General Biology of Bacteria and the General Methods of Bacteriology; II, Infection and Immunity; III, Pathogenic Microorganisms; IV, The Rickettsia Diseases; V, The Spirochetes; VI, Medical Mycology: Molds, Yeasts, Actinomycetes, and Pathogenic Fungi; VII, Diseases Caused by Ultramicroscopic Viruses, the Exanthemata, and Diseases of Uncertain Etiology; VIII, Outline of Diagnostic Methods of Medical Protozoology; IX, Technical Methods of Bacteriology, Immunology, and Serology.

The revision has been thorough and extensive and embodies consideration of all the many and marked advances which have been made within the last ten years in the field of bacteriology and immunology and includes a discussion of the contributions made in 1933 and the early part of 1934.

As before, the book constitutes an outstanding contribution in this field and one without which no reference library can be complete.

It can be enthusiastically recommended without reserve and, without doubt, will receive the same general approval as its predecessors.

\*Practical Methods in Bacteriology. By F. C. Koch, Professor of Physiological Chemistry, University of Chicago, Cloth, pp. 282. 17 figures. William Wood & Co., Baltimore, Md.

†A Textbook of Bacteriology. By Hanz Zinsser, M. D., Professor of Bacteriology and Immunology, Harvard University; and Stanhope Bayne-Jones, M.D., Professor of Bacteriology, Yale University Medical School. Ed. 7, cloth, pp. 1226, 174 illustrations. D. Appleton-Century Co., New York.

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## EDITORIALS

### Barnacles

**H**UMAN psychology perhaps to some extent may be reflected in its proverbs so that the time-worn sayings concerning the silver lining and the ill wind that, despite itself, blows some one some good all may justify the dictum that "hope springs eternal in the human breast."

Certain it is that not infrequently in the history of civilization great catastrophes, extraordinary plagues and similar events, regarded at the time as crushing and devastating blows to fortune, have been instead the starting points for tremendous advances and discoveries and have initiated a cycle of events productive of far-reaching benefits to humanity.

If history, as it is reputed to do, again repeats itself, perhaps the depression into which the world at large has been plunged for some years past and from which it shows faint but indubitable signs of beginning to emerge, may in its turn be productive of direct and indirect benefits.

It was inevitable under the circumstances that the social world should, in the face of its apparent collapse, take stock of the mechanism which holds it together and, as it were, makes it go; and very naturally medicine, in which term is included all that has to do with the management and control of disease and its congeners, has not escaped a critical glance.

Community responsibility for the care of the sick is an essential part of the development of civilization, for the discharge of which a huge structure has developed, ranking in importance, not only because of its essential necessity but also because of its economic magnitude and ramifications, with any of all those of which modern civilization can boast.

Hospitals are an integral part of this structure and, although not commonly regarded as "Big Business," properly belong in this category; for the seven thousand hospitals in the United States in round figures represent a capital investment of more than three billions of dollars, a capital sum exceeded by but few of the great industries.

It is entirely probable that there is little general appreciation of the fact that the average one hundred bed hospital represents an average capital investment of one million dollars and that the average investment per bed in accordance with the size and type of hospital, for acute or chronic diseases or including research facilities, varies from \$2,500 to more than \$10,000.

On the basis of the investment involved hospitals must be regarded as a business, "Big Business," the business of medical care, and the fact that this business ship within recent years has been laboring rather heavily in a heavy, stormy economic sea, as it were, has focussed attention upon this structure and the proposal by many individuals and groups of various measures to allay the storm and set the vessel once more on an even keel.

Oceans of ink have already been spilled in the discussion of what has popularly become familiar as "the cost of medical care" and the end of the avalanche of words and windy dispute is far from near, nor will it be found herein, for the subject is not to be dismissed within the limits of an editorial. Nor is any discussion here intended of the relation of hospital costs to the general costs of illness, important as it is. Those are matters deserving abler pens; these are but casual observations.

Above, the hospital structure with a striking lack of originality has been likened to a ship and a meager acquaintance with matters nautical—gathered, it must be confessed, from perusal of such authorities as Marryatt, the varied tales of Conrad, *Moby Dick*, and the reading (repeatedly) of *Treasure Island*—allow us to state with some confidence that when the good ship no longer shows her accustomed speed but, instead, merely lumbers along, wallowing ungracefully, the scraping from the bottom of barnacles and accumulated débris was a very common custom apparently productive of the desired result.

Can it be that hospitals in the course of time have accumulated any barnacles? Can it be that this huge structure has drawn to itself by custom or habit any accretions not perhaps essential per se to its efficiency or existence?

The business of hospitals, as already said, is the business of medical care and hence indissolubly associated with the practice of medicine and the physi-

cian. And physicians of late have had it brought rather forcibly to their attention that to some extent, varying under different circumstances and in different localities, the practice of medicine seems to have passed or to be passing from the hands of the physician into the hands of the hospital.

Now, while it has been possible in the past, and is still possible in the present, to carry on the business of taking care of the sick without the hospital, it is perhaps sometimes minimized if not overlooked that the hospital without the physician is rather a helpless if not useless institution. It is conceivable that there are hospital boards and perhaps, even superintendents to whom such an idea would appear preposterous, and it is not strange that this should be so when one pauses to consider how little, relatively speaking, the physician has to do with the running of hospitals.

True, as a dispensary worker he gives untold hours of his time freely and without remuneration to the care of patients, not a few of whom are without moral right or justification for such free treatment, and after often a lengthy period of service in this capacity, he graduates to the dispensing of free care under like circumstances in the wards, and sometimes, as at present, there comes some vague wonderment as to just how all this came about and whether or not he may not thereby be committing a sort of slow economic hara-kiri.

If, as is not at all beyond the bounds of possibility, the practice of medicine is to become a function of the social organization either permanently or temporarily until such time as the futility of such a program, despite its theoretical advantages, becomes evident in practice, the physician may well wonder just what place will be his in relation to the hospital.

Although the business of the hospital is the business of taking care of the sick, the physician is seldom indeed found directing its destiny in that respect, for custom seems to have decreed that hospital superintendents are most often graduate nurses. Just why this should be so; what basis there is for assuming that the training of a nurse implies executive ability or the ability to supervise rather than to assist in the care of the sick, is, it must be confessed, somewhat difficult to ascertain.

Yet the fact remains that perhaps a majority of hospitals of average size have nurses as superintendents, not infrequently directing the Board of Managers as well as the hospital and casting a supervisory eye over the activities of the staff. And, as some cynic once remarked: Hospitals are sometimes mismanaged because they are Miss managed.

If, in his extremity, the physician turns to the field of anesthesia, certainly an important one, properly a specialty of medicine and so largely regarded in England, here, too, he finds the nurse rather firmly ensconced.

There is no place for him on the Board of Managers, for it is only very recently that Staff representation has been granted as a favor.

He may strongly suspect that this or that free patient is well able to pay for treatment and belongs in the doctor's office rather than the dispensary, but the nurse or the lay social service worker has decreed otherwise, and that's that!



He finds himself arraigned and accused of grave responsibility for the high costs of medical care, and finds it somewhat difficult to reconcile the accusation with the fact that the money earmarked for payment of his bill has been swallowed up in payment of the hospital's.

And as the economic storm rages about him, he finds himself working for the hospital, carrying on the practice of medicine for it and nourishing thereby a host of lay personnel. Neither skipper, mate, nor even bos'n, small wonder that he wonders whether it were not better to be merely another barnacle.

—R. A. K.

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### The Anemia of Pregnancy

WHILE the fact that pregnancy may be accompanied by a varying degree of secondary anemia is more or less commonly recognized, it is only within recent times that, possibly as an aftermath of studies in prenatal care, much attention has been paid to its presence or much effort expended upon the determination of its degree.

We refer, not to the severe hemolytic anemia, sometimes spoken of as the pernicious anemia of pregnancy, but to the definite secondary, though not always marked anemia which seems to be the result of pregnancy per se.

The pernicious or hemolytic anemia of pregnancy is encountered during the later months of pregnancy and the puerperium. Insidious in onset, it may reach a marked degree without the production of marked symptoms and thus may escape detection until attention is drawn to it by symptoms closely resembling those seen in true pernicious anemia.

The hemolytic anemia of pregnancy is the result of the action of a synectial hemolysin arising in the ectodermal cells of the chorion. Under normal conditions as pregnancy progresses an antihemolysin is formed in the maternal blood but when, for any reason, its formation is inhibited, the hemolytic anemia of pregnancy is the result.

Fortunately, in view of its rather high mortality, this form of anemia in pregnancy is rather uncommon.

The less marked but still definite secondary anemia of pregnancy, on the other hand, is very common and of definite significance although often overlooked when cytologic examinations of the blood are neglected as a part of the prenatal survey.

The findings of practically all observers who have reported upon this subject present a remarkable conformity.

Bland and Goldstein,<sup>1</sup> in a study of 50 cases, found a hemoglobin content ranging from 42 to 78 per cent (Dare) and erythrocyte counts of from 2.36 to 3.98 million cells per cubic millimeter.

In a second study of 300 cases, 100 being private cases, Bland, Goldstein and First,<sup>2</sup> found red cell counts below 3.5 million in 33.3 per cent of cases during the first two trimesters of pregnancy, 55.7 per cent in the third trimester, and 26 per cent during labor.

Galloway<sup>3</sup> in a study of 382 cases found average hemoglobin values (Sahli) of 73, 69, and 66 per cent for the first, second, and third trimesters respectively, and erythrocyte counts of 4.05, 3.94, and 3.87 millions per cubic millimeter evidencing both a chlorotic type of anemia as well as a definite progression as the pregnancy advanced.

Practically the same findings are reported by Kilduffe<sup>4</sup> in 300 cases as shown in Table I.

TABLE I

| DURATION OF<br>PREGNANCY,<br>MONTHS | NO. CASES | AVERAGE Hb.<br>(HADEN) |          | AVERAGE R.B.C.<br>MILLIONS PER<br>C. MM. |
|-------------------------------------|-----------|------------------------|----------|------------------------------------------|
|                                     |           | GM.                    | PER CENT |                                          |
| 1 - 3                               | 12        | 10.07                  | 73       | 4.50                                     |
| 4 - 6                               | 72        | 9.93                   | 72       | 3.73                                     |
| 7 - 9                               | 216       | 9.10                   | 66       | 3.73                                     |

It is unnecessary to cite the many other studies available, as all present comparable findings so that it may be accepted as proved that an anemia secondary to pregnancy per se is present in from 50 to 60 per cent of all cases, which shows a tendency to be somewhat chlorotic in type and a definite tendency to progress *pari passu* with the progression of the pregnancy.

The mechanism responsible for its production has been the subject of much discussion, many different theories having been advanced, none of which, however, have been definitely proved.

Thus, it has been attributed to hydremia producing a decreased iron content of the corpuscles, which would account for its chlorotic type; to poor hygiene or the presence of such diseases as nephritis, syphilis, and tuberculosis; to the continuation of a preexisting anemia; to focal infections; and to the expression of an "individual disposition" in accordance with the general physical well-being of the individual patient.

As stated, however, none of these conceptions has been susceptible of clear-cut, definite or indisputable proof.

The possible relation of this secondary anemia of pregnancy to the presence of the syncytial hemolysin held to be responsible for the hemolytic anemia mentioned above but inhibited in the case of the secondary by the later production of an antihemolysin is one of the oldest theories but, like all the others, still remains but a theory while its occurrence in the higher classes of patients, usually seen as private cases, robs environment factors of all significance.

Many investigators of this question assert that the anemia spontaneously disappears after delivery but many others are unable to find conclusive evidence of the correctness of this contention.

The matter still remains a problem awaiting solution, the present status of which may be thus summarized:

1. Pregnancy, per se, produces a secondary anemia somewhat chlorotic in type which progresses *pari passu* with the progression of pregnancy.

2. No satisfactory or demonstrable etiologic mechanism has yet been found.

3. In view of these facts, cytologic blood studies should be a part of routine prenatal care.

4. All patients with a hemoglobin below 75 per cent and erythrocyte counts under 3.5 million should be particularly observed and therapeutic measures instituted immediately upon the discovery of the deficiency. It is advisable also that such patients be watched for several months after delivery for evidence of bone marrow changes.

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—R. A. K.

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## CORRESPONDENCE

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### Another Simple Rotary Inoculating Table

*To the Editor:*

Whether one has many or few Petri plates to stroke, a rotary table gives very satisfactory results and is a great convenience.

Quite a number of designs of such a piece of apparatus have appeared in the recent literature, requiring for construction the services of anyone from a blacksmith to a Swiss watchmaker.

We have found that the simplest and most satisfactory method of constructing such a machine is to take a two-dollar bill and go out and buy an old discarded portable Victrola. (Since the advent of Radio, one can sometimes get two dollars for taking the machine away.) If the reader cannot improvise from several old records ("She Knows Her Onions" or "On the Beach at Waikiki" preferred) and some thumb tacks, a means of holding the Petri plate on the revolving turntable, he should not be trusted with a platinum loop. With the brake the speed may be adjusted to suit the bacteriologist's artistic temperament.

We have used our machine for four or five years with utter satisfaction.

ERIC A. FENNEL, M.D.

HONOLULU, HAWAII, APRIL 10, 1934.

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